ON AZULENE AND ITS NON-PREEXISTENCE IN MILFOIL

Thesis submitted for the Degree of
Doctor of Philosophy

by

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History. In 1863 Piesse[1)] made a study of the blue constituent of several volatile oils[2)] which he termed azulene, "from azure-blue". Gladstone,[3)] in 1864 published his observations[4)] on the same subject and gave the name coerulein[5)], from the Latin, coeruleus, blue, to the compound in question. The blue color of volatile oils or of higher fractions thereof had previously been observed[6)]. Until quite recently these blue fractions were supposed to contain oxygen, hence were impure, i.e. contaminated with oxygenated constituents. In 1915, however, Sherndal[7)] isolated the pure hydrocarbon, to which he assigned the formula $C_{15}H_{18}$, by regenerating it from its sulphuric acid and phosphoric acid addition products. Since then blue hydrocarbon material has been studied by Augsburger[8)] (1915), R.E. Kremers[9)] (1923), Ruzicka and Rudolph[10)] (1926), Ruhemann and Lewy[11)] (1927) and Asahina and Nakanishi[12)] (1928 and 1932). Ruzicka differentiates several azulenes.

1.) Pharm. Journ., 23 (1864), p. 277.

2.) Piesse separated the blue fractions of the following oils:- Chamomile, Wormwood, Patchouly (Pogostemon patchouly), Bergamot (Citrus bergamia) and Ceylon Lemon-grass (Andropogon Schoenanthus).

3.) Journ. Chem. Soc., 17 (1864), p. 1. (Abstr. in Chem. Centralbl., 35, p. 575.)

4.) Gladstone fractionated the volatile oils of:- Wormwood (Artemisia Absinthium), Matricaria Chamomilla, and Achillea Millefolium.

5.) Gladstone objected to the name azulene because the term azuline had already been applied to a coal tar dye.

6.) The earlier observations on blue oils or fractions thereof are herewith arranged in their chronological order.

Year	Plant	Observer and ref.
1839	Achillea Millefolium	Forcke, Arch.d.Ph., 67, p. 177.
1844	Chamomille oil	Borntraeger, Ann., 49, p. 243.
1861	Galbanum resin	Moessmer, Ann., 119, p. 262.
1863	Chamomile, Wormwood, Ceylon Lemon-grass, Patchouly, Bergamot,	Piesse, Ph. J., 23, p. 277.
1864	Wormwood, Matricaria Chamomilla, A. millefolium	Gladstone, J. Chem. Soc., 17, p. 1.

Year	Plant	Observer and ref.
1871	Chamomile	Kachler, Ber., 4, p. 36.
1874	Acorus Calamus	Kurbatow, Ann., 173, p. 4.
1876	Guaiaci Peruviana aromatica	Kopp, Arch. d. Ph., 209, p. 193.
1878	Valerina officinalis	Bruylants, Ber., 11, p. 456.
1883	Chamomile, Wormwood, Milfoil	Hock, Arch. d. Ph., 221, p. 17.
1888	Turnera diffusa	Ber. Schimmel and Co., April, 1888, p. 44.
1889	Angelica anomala	Ber. Schimmel and Co., April, 1889, p. 3.
1891	Asafoetida oil	Semmler, Arch. d. Ph., 229, p. 1.
1892	Cedrela	Ber. Schimmel and Co., April, 1892, p. 41.
1893	Achillea coronopifolia	Report Schimmel and Co., April, 1893, p. 72.
1894	Andropogon Schoenanthus	Barbier and Bouveault, C.r., 119, p. 281.
1894	Anethum graveolens,	Report Schimmel and Co., Oct., 1894, p. 32.
1895	Sagapen oil, Jap. valerian, Wormwood, Milfoil, Asafoetida Galbanum, Inula Helenium, Valerina officinalis,	Tschirch and Hohenadel, Arch. d. Ph., 233, p. 259.

Year	Plant	Observer and ref.
1895	Cubeb oil	Umney, Ph. J., 54, p. 951.
1896	Piper Lowong	Peinemann, Arch. d. Ph., 234, p. 242.
1899	Aralia Nudicaulis	Alpers, Am. J. Ph., 71, P. 370.
1899	Ferula opopanax	Tschirch and Knite, Arch. d. Ph., 237, p. 256.
1902	Milfoil	Aubert, J. Am. Chem. Soc., 24, p. 778.
1902	Asarum canadense	Power and Lees, Jour. Chem. Soc., 81, p. 592.
1907	Milfoil	Sievers, Ph. Rev., 25, p. 215.
1908	Artemisia arborescens	Report Schimmel and Co., Oct., 1908, p. 145.

7.) J. Am. Chem. Soc., 37 (1915) pp. 167 and 1537.

8.) Science (New Series) 42 (1915) p. 100.

9. J. Am. Chem. Soc., 45, (1923) p. 717.

10.) Helv. Chim. Acta, 9 (1926) p. 118.

11.) Berichte, 60(1927) p. 2459.

12.) Journ. Ph. Soc. Japan, 48 (1928) p. 1; ibidem, 52 (1932) p. 2.

Occurence:- The presence of azulene had been identified in plants in comparatively few cases but its existance is indicated in a great number of oils by a blue or green color. In the following table the families are arranged according to the system of Engler and Prantl. The numbers are given in order to indicate how many families have thus far been observed to yield blue oils or blue fractions of oils.

4. Pinaceae

Cryptomeria japonica, Don.	Blue fraction1)
Pinus monticola, Dougl.	Blue fraction2)

13. Gramineae

Andropogon Schoenanthus, L.	Blue fraction3)

17. Araceae

Acorus Calamus, L.	Blue fraction4)

29. Zingiberaceae

Alpinia officinarum, Hance	Greenish-yellow5)

35. Piperaceae

Piper nigrum, L.	Greenish6)
Piper Lowong, Bl.	Blue fraction7)
Piper Cubeba, L.	Azulene8)

49. Aristolochiae

Asarum caudatum	Blue fraction9)
Asarum canadense, L.	Blue10)

71. Lauraceae

Laurus Camphora, L.	Azulene11)
Neotandra Puchury-major, Nees	Blue fraction12)
Neotandra Puchury-minor, Nees	Blue fraction12)

98. Rutaceae

Ruta graveolens, L.	Blue fraction13)

101. Meliaceae

Cedrela	Light blue14)
Dysoxylon acutangulum, Miq.	Azulene15)

104. Euphorbiaceae

Cathetus fasciculata, Lour.	Bluish green16)

124. Malvaceae

Gossypium	Blue fraction17)

139. Turneraceae

Turnera diffusa, Willd.	Blue fraction18)
Turnera aphrodisiaca, Ward.	Blue fraction18)

158. Myrtaceae

Leptospermum scoparium, Forst.	Azulene19)

165. Araliaceae

Aralia nudicaulis, L.	Blue fraction20)

166. Umbelliferae

Petroselinum sativum, Hoffm.	Yellowish-green21)
Angelica refracta, Schmdt.	Blue fraction22)
Angelica anomala, Lall.	Blue fraction22)
Ferula foetida, Reg.	Blue fraction23)
Anethum graveolens, L.	Greenish-blue24)
Pimpinella nigra, Willd.	Light blue25)
Siler trilobum, Scop.	Azulene26)

170. Ericaceae

Ledum groenlandicum, Retz.	Blue fraction27)

190. Labiatae

Mentha aquatica, L.	Yellowish-green[28]
Mentha javanica, Bl.	Light green[29]
Pogostemon Patchouly, Pell.	Blue[30]
Ocimum sanctum, L.	Green[31]
Lophanthus anisatus, Benth.	Brownish-green[32]
Salvia officinalis, L.	Greenish-yellow[33]
Calamintha Nepeta, Savi	Greenish-yellow[34]
Origanum Majorana, L.	Greenish-yellow[35]
Perilla nankinensis, Dcne.	Greenish[36]

202. Rubiaceae

Nelitris, Gaertn.	Yellowish-green[37]

203. Caprifoliaceae

Sambucus nigra, L.	Yellowish-green[38]

205. Valerianaceae

Valeriana officinalis, L.	Yellowish-green[39]
Valeriana officinalis, L., var. angustifolia, Miq.	Blue[40]

211. Compositae

Eupatorium triplinerve, Vahl	Light green[41]
Inula Helenium, L.	Blue fraction[42]
Parthenium argentatum, Gray	Greenish-yellow[43]
Ambrosia artemisifolia, L.	Deep green[44]
Osmites Bellidiastrum, L.	Yellowish-green[45]
Achillea Millefolium, L.	Azulene[46]
Achillea Coronopifolia, Willd.	Blue[47]

Achillea nobilis, L.	Blue[48]
Achillea moschata, Jacq.	Dark blue[49]
Solidago nemoralis, Ait.	Olive green[50]
Chrysanthemum japonicum, Thunbg.	Green[51]
Tanacetum boreale, Fisch.	Greenish brown[52]
Matricaria Chamomilla, L.	Dark blue[53]
Matricaria Parthenium, L.	Dark green[54]
Artemisia Herba-alba, Asso	Greenish-yellow[55]
Artemisia arborescens, L.	Azulene[56]
Artemisia Absinthium, L.	Azulene[57]
Artemisia maritima, L.	Dark green[58]
Artimisia frigida, Willd.	Greenish[59]
Artemisia Ludoviciana, Nutt.	Greenish-yellow[60]
Artemisia pontica, L.	Blue[61]

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2.) Lehman and Lynn, J. A. Ph. A., 19 (1930) p. 1187.

3.) Barbier and Bouveault, C.r., 119 (1894) p. 281.

4.) Kurbatow, Ber., 6 (1873) p. 1210.

5.) Gildemeister, Die Aeth. Oele, 3rd. ed., Vol. II, p. 434.

6.) Eberhardt, Arch. Ph., 225 (1887) p. 515.

7.) Peinemann, Arch. d. Ph., 234 (1896) p. 242.

8.) Sherndal, J. Am. Chem. Soc., 37 (1915) p. 1537.

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24.) Report of Schimmel and Co., Oct. (1894) p. 32.

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28.) Ber. Schimmel and Co., Oct. (1889) p. 55.

29.) Wielen, Apoth. Ztg., 19 (1904) p. 930.

30.) Gladstone, J. Chem. Soc., 17 (1864) p. 3.

31.) Bacon, Philippine Journ. Sci., 5, A(1910) p. 261.

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33.) Gildemeister (Kremers), The Volatile Oils, 2nd. ed. Vol.III, p. 455.

34.) Report of Schimmel and Co., Oct. (1906) p. 14.

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Occurence:- The pre-existance of azulene in the plant was first questioned by Tschirch and Hohenadel in 1895[1), when they observed that sagapen yielded a yellow oil upon extraction with petroleum ether and that this oil became blue during fractionation. Not knowing whether the formation of the blue substance was due to the exposure of the volatile oil extracted with petroleum ether or to resin extracted at the same time, they prepared a resin-free volatile oil by steam distillation. This also was faintly yellow and only upon fractional distillation involving high temperatures, viz. abt. 200°, did they obtain a blue fraction. They, therefore, arrive at the conclusion that "without doubt, the blue oil is a pyrogenic decomposition product".2) In 1917, however, Tschirch expresses himself as still in doubt whether the azulene is formed during the process of distillation.3)

Herzenberg and Ruhemann made a similar investigation in 1927 4). They found that chamomille yielded only a small amount of a yellow oil to petroleum ether but that the extracted plant yielded a blue oil upon steam distillation. From this they concluded that azulene did not pre-exist in the plant and that its formation was from sesquiterpenes by fermentative action especially since they had isolated a sesquiterpene which yielded a blue color by dehydrogenation. However, the experiment performed does not support this conclusion. If the azulene were formed from sesquiterpenes, it would not then be obtained from the extracted marc, from which the sesquiterpenes had been removed. Further, fermentative action would not be expected during steam distillation, where the temperature is much above the thermal death point of enzymes. Nor is dehydrogenation likely to take place during steam distillation.

The existence of azulene in the plant may more logically be explained by the assumption of an acid addition product since it is known that azulene readily forms such a product which is not soluble in petroleum ether. The union of azulene with either phosphoric acid or an acid phosphate would presumably give a compound which could not be extracted with petroleum ether but which could be decomposed by the action of steam.

Another hypothesis which may explain the presence of combined azulene, i.e. in a form which cannot be extracted with petroleum ether, is that it may exist in glucosidal combination. We usually consider glucosides to be ether like products of the union of a sugar and an alcohol. It is barely possible that a hypothetical colorless alcohol, $C_{15}H_{17}OH$, when set free and at the higher temperatures involved in fractionation loses a molecule of water thus yielding the blue hydrocarbon. This hypothesis is based on a possible analogy with the formation of a terpene, $C_{10}H_{16}$. observed by Kayser[5)] in 1884 who obtained it upon the hydrolysis of the glucoside, picrocrocin. The presumption in this case is that a terpene alcohol results upon hydrolysis of the glucoside and that under the conditions of the experiment the alcohol breaks up into the terpene and water.

The experiment of Herzenberg and Ruhemann has been repeated upon a larger scale and the results found are in accordance with their investigations.

The material used was collected in June, 1930, from the garden of the Wisconsin Pharmaceutical Experiment Station and from land southwest of Madison, under the direction of Professor W.O. Richtmann. Having been air dried, the inflorescences were carefully removed and ground in a Grumbach mill.

I. Extraction and distillation of milfoil flowers

4988 gms of the ground milfoil flowers were extracted with petroleum ether in the Lloyd extractor. The flowers thus extracted were exposed so as to allow any adhering petroleum ether to evaporate. They were transfered to a 60 liter Lentz copper still and subjected to steam distillation. The periods of distillation and the amount of aqueous distillate obtained in each are herewith tabulated:.

Period	Time	Amount
1st day	2 hrs.	7 gal.
2nd "	3 "	10 "
3rd "	2 "	12 "
4th "	0 "	0 "
5th "	0 "	0 "
6th "	0 "	0 "
7th "	5 "	15 "
8th "	1½ "	5 "
9th "	4½ "	20 "
10th "	1 "	5 "
Totals	19 hrs.	73 gal.

The distillation was discontinued when no more color could be extracted from the distillate with ether. The aqueous distillate which gave no acid reaction with litmus paper, was shaken with ether, and the ethereal solution separated. The solvent was recovered by distillation and there remained 4.8 cc (0.096 per cent) of a deep blue oil, which had a density of 0.9516 at 25°. This oil was treated with phosphoric acid, and the azulene-phosphoric acid compound hydrolized with water. The liberated azulene was extracted with ether and after the removal of the solvent, 0.5 cc of azulene remained (0.01 per cent.)

The petroleum ether extract resulting from the percolation of the flowers was distilled under reduced pressure to remove the solvent. The residue thus obtained weighed 186 gms. When steam distilled, 12 cc (0.24 per cent) of a light blue oil separated from the aqueous distillate. It had a density of 0.9105 at 25°. The oil was fractionated under atmospheric pressure with the following results:

Temp.	Amount
-170°	0.3 cc.
170-180°	2.0 "
180-190°	2.5 "
190-200°	2.1 "
200+°	4.2 "

II. Extraction of the azulene compound

1. Hot extraction with chloroform.

100 gms of the ground flowers were placed in a continuous extractor and exhausted with chloroform. The heat of the vapors was sufficient to keep the small percolator warm during the extraction. The solvent was removed from the extract by distillation under reduced pressure and the extract washed with solvents, resulting in the following products: A.) a petroleum ether extract, B.) an ether extract, and c.) a residue.

A.) The petroleum ether extract was steam distilled and the distillate washed with ether. The ethereal solution was colored blue.

B.) The ether extract was steam distilled and the distillate washed with ether. The ethereal solution was also colored blue.

C.) The residue was steam distilled and the distillate washed with ether, yielding no blue color.

D.) The extracted flowers were distilled with steam and the aqueous distillate yielded no blue color when washed with ether.

2. Cold extraction by maceration.

1000 gms of the dried flowers were extracted by maceration with chloroform at room temperature. The chloroform was removed by distillation under reduced pressure. This extract was treated as in the previous experiment and resulted in similar products:

A.) The petroleum ether was removed by distillation under reduced pressure. The extract was steam distilled and yielded 3.4 cc (0.34 per cent) of a yellow oil.

B.) The ether extract was steam distilled after the removal of the ether. The distillate when washed with ether, yielded a blue color.

C.) The residue was steam distilled and the distillate yielded a blue color.

D.) The extracted flowers, when steam distilled, yielded a faint blue color.

3. Extraction by percolation.

Two samples of 1000 gms each of the ground flowers were packed in a percolator and extracted with chloroform.

The chloroform was removed from the extract by distillation under reduced pressure. The extracts were treated as in the previous experiment:-

A.) The petroleum ether was removed by distillation under reduced pressure. The extracts were steam distilled and 3 cc and 3.1 cc of a yellow oil resulted.

B.) The ether was removed by distillation under reduced pressure and the extracts steam distilled. The distillate from both extracts yielded a blue color.

C.) The residues were steam distilled and both yielded a blue color.

D.) The extracted flowers were distilled with steam and no blue color was obtained.

4. Preparation of the chloroform extract.

15,400 gms of the ground flowers were packed in percolators and extracted with chloroform. The chloroform was removed by distillation under reduced pressure and 1520 gms. of extract resulted. The extract was washed with petroleum ether, resulting in two products: A.) 577 gms. of a petroleum ether extract and 790 gms of residue.

III. The azulene compound

The 790 gms of extract previously obtained contained the azulene compound and were used in the following experiments.

1.) Determination of the inorganic constituents.

a.) 0.8277 gms. of the extract yielded no ash.

b.) 1.6176 gms. of the extract yielded 0.0001 gms. ash.

These results indicate the improbability of an acid addition product since azulene is not known to add to any organic acid except formic, with which it forms a liquid and easily hydrolized compound.

2.) Acid hydrolysis.

If azulene is contained in the molecule in glucosidal combination, hydrolysis with acid should liberate it from the accompanying sugar molecule, which could be detected. The extract reduced Fehling's solution before hydrolysis and after hydrolizing with dilute hydrochloric acid, the amount of copper oxide was increased but constant values could not be obtained.

The extract was hydrolized for ten hours with 5 per cent sulphuric acid. After the reaction mixture was filtered it was divided and made neutral with sodium hydroxide. One half of the solution was extracted with ethyl acetate. Both portions were then treated with Fehling's solution. The untreated portion did [redacted] reduce Fehlings solution. The portion washed with ethyl acetate did not reduce the copper solution.

Therefore, it was concluded that the extract contained no sugar and did not yield sugar by acid hydrolysis.

3. Ester Value.

The ester value of the extract was determined in order to obtain an indication of the possibility of alkaline hydrolysis.

a.) 6.4204 gms. of the extract required 0.379 cc of normal potassium hydroxide for neutralization, corresponding to an acid value of 61. When heated for one half hour, the sample reacted with 1.94 cc of normal potassium hydroxide, corresponding to a saponification value of 261. Ester value = 200.

b.) 0.4046 gms. of the extract required 0.4 cc of normal potassium hydroxide for neutralization, corresponding to an acid value of 66. When heated, it reacted with 1.94 cc of normal potassium hydroxide, corresponding to a saponification value of 272. Ester value = 206.

4. Alkaline hydrolysis

The extract was treated for an hour with sodium hydroxide solution without heat. The mixture was filtered on a force filter and the residue washed with water. The filtrate was made acid with dilute hydrochloric acid, added slowly and with continuous stirring. The resulting precipitate was separated on a force filter and washed with water. The filtrate was distilled to dryness and the residue extracted with alcohol, which was removed by distillation. The results of the hydrolysis are shown in the accompanying table:

Reagent	Extract	Insol. sub.	Sol. sub.	Residue
5 %NaOH	4 gm.	0.05 gm.	0.7 gm.	-------
5 % "	10 "	0.3 "	4.5 "	3.0 Gm.
5 % "	10 "	0.1 "	2.0 "	7.2 "
5 % "	10 "	0.1 "	4.3 "	3. 5 "
2 % "	10 "	2.7 "	1.2 "	5. 0 "
2 % "	10 "	2.7 "	1.9 "	4. 5 "
2 % "	10 "	3.7 "	1.0 "	4. 5 "

The acid precipitated substances of the first four experiments were bulked and neutralized with sodium hydroxide. This sodium salt was used to prepare the barium and silver salts. 5 gms. of the sodium salt yielded 2 gms of the barium salt, which was analyzed for barium content.

a.) 0.9198 gms. of the barium salt yielded 0.0968 gms. of barium carbonate corresponding to 7.32 per cent of barium.

b.) 1.0230 gms. of the barium salt gave 0.1070 gms. of barium carbonate, corresponding to 7.38 per cent of barium.

5 gms. of the sodium salt were treated with silver nitrate and yielded 5.5 gms of silver salt, which upon analysis yielded the following results:

a.) 0.9760 gms. of the silver salt gave 0.2395 gms. of silver, corresponding to 24.5 per cent of silver.

b.) 1.0945 gms. of silver salt gave 0.2690 gms. of silver, corresponding to 24.57 per cent of silver.

Attempts to form oximes or acetyl derivatives with other products of the hydrolysis yielded no results.

5. Carbonyl oxygen.

a.) reaction with phenyl hydrazine. 0.5 gms. of the extract were dissolved in alcohol. 2 cc. of acetic acid and 5 cc of phenyl hydrazine were added. The mixture was warmed for 10 minutes. Upon cooling, small crystals of phenylhydrazine acetate, m.p. 127°, settled out and were filtered off. The mother liquor yielded an amorphous product with no characteristic melting point.

b.) Reaction with hydroxylamine. 2 gms. of the extract were dissolved in alcohol and treated with 2 gms. of hydroxylamine hydrochloride and sodium carbonate. Upon standing, a grayish green amorphous precipitate formed. This precipitate was filtered off and treated with hydrochloric acid. The resulting solution gave no reduction of Fehling's solution.

6. Hydroxyl groups.

a.) Acetylation. 5 gms of the extract were heated with acetic anhydride and sodium acetate for one hour. The product was washed with sodium carbonate solution, then with water and dried. The saponification value of this product could not be determined because of the color.

b.) Benzoylation. 13.5 gms. of the extract were dissolved in 50 cc. of pyridine and cooled well. 15 cc. of benzoyl chloride were added in small portions, shaking after each addition. White crystals separated which were filtered off on a force filter and washed with pyridine. The platinum double salt of these crystals melted at 240° (pyridine hydrochloride). The filtrate was diluted with ether. 2.2 gms. of an insoluble residue remained, which when treated with sodium hydroxide gave an odor of pyridine. The ethereal solution was washed with dilute hydrochloric acid, with sodium carbonate solution and with water and the ether removed by distillation under reduced pressure. 14 gms. of the ester were obtained.

a.) 1.0860 gms. of the ester reacted with 4.88 cc of normal sodium hydroxide, corresponding to a saponification value of 178.

b.) 0.9885 gms. of the ester reacted with 4.39 cc. of normal sodium hydroxide, corresponding to a saponification value of 179.

7. Oxidation with nitric acid.

Since the action of nitric acid upon azulene is not known it would render the situation less complicated if the azulene could be removed from the molecule before oxidation. Steam distillation of the extract yielded the azulene very slowly, some blue color being obtained after 70 hours of distillation. Therefore, other means for the removal of the azulene were sought.

5 gms. of the extract were mixed with 300 cc. of water and placed in a heavy walled, tightly stoppered bottle and heated on an oil bath at 150° for eight hours. The contents of the bottle were then steam distilled. The first distillate yielded a deep blue color and as distillation continued less and less of the color was obtained. However, after 60 hours of distillation some blue color was still obtained.

Acid hydrolysis using 15 per cent hydrochloric acid in amyl alcohol solution6) yielded no azulene when the contents of the flask were steam distilled. The same reaction was tried under pressure with no different results. Therefore, the attempts to remove azulene from the molecule were abandoned.

Oxidation of the extract was conducted in the following manner: 5 gms. of the extract were mixed with 20 cc. of nitric acid, added slowly while the mixture was kept well cooled. The mixture was then allowed to warm slightly, almost to room temperature. As soon as brown fumes were evolved the mixture was cooled in an ice bath so that the reaction proceeded slowly. When no more brown fumes were evolved with the mixture at room temperature, the reaction was complete. An insoluble substance rose to the surface of the liquid and was separated and washed with water. The reaction mixture was diluted with water and a flocculent precipitate resulted which was filtered off and washed with water. The bright yellow aqueous liquids were united and saved for further investigation.

The results of four such experiments are given in the following table.

Extract	Insol.Sub.	Solub.Sub.	Total
5 gms.	-------	1.19 gms.	-----
5 "	3.89 gms.	0.45 "	4.34 gms.
5 "	1.6 "	1.6 "	3.2 "
5 "	1.6 "	1.22 "	2.82 "

The products were combined and washed with boiling alcohol. This procedure yielded 1.4 gms. of a substance insoluble in alcohol, 1.2 gms. which precipitated from the hot alcohol and 6 gms. of an amorphous residue. This residue was acid to litmus, soluble in sodium hydroxide solution from which it was precipitated by acid. From the sodium salt a barium and a silver salt was prepared.

1.) 0.3330 gm. of the barium salt yielded 0.0190 gms. of barium carbonate corresponding to 4.1 per cent of barium.

2.) 0.7100 gm. of the barium salt yielded 0.5000 gms. of barium carbonate, corresponding to 4.7 per cent of barium.

3.) 0.8080 gms of the silver salt yielded 0.1762 gms of silver, corresponding to 21.8 per cent of silver.

4.) 0.7000 gms of the silver salt yielded 0.1470 gms of silver, corresponding to 20.0 per cent of silver.

The aqueous liquids from the oxidation mixture were distilled and resulted in a clear, acid distillate and a yellow, acid residue from which nothing solid separated. The distillates were combined and neutralized with barium carbonate. The mixture was filtered and the residues tested for organic matter. None was found present. The aqueous portion was concentrated and the crystals obtained did not char. This excludes the possibility of volatile acids resulting from the oxidation.

The residues were combined and neutralized with barium carbonate. The excess barium carbonate was filtered off and gave no test for organic matter. The aqueous portion when evaporated to dryness was found to contain organic material. A test for picric acid could not be obtained.

1.) Arch. d. Ph., 233 (1895) p. 259.

2.) Ibidem, p. 279.

3.) Tschirch, Handbuch d. Pharmakognosie, Vol. II, p. 964.

4.) Ber., 60 (1927) p. 2464.

5.) Ber., 17 (1884) p. 2228.

6.) Power and Salway, J. Chem. Soc., 103 (1913) p. 399.

Isolation. Early investigators attempted the isolation of azulene by fractionation but this method failed to yield results even with oils of intensely blue color since the amount of azulene is relatively small and mixed with other high boiling constituents, e.g. the sesquiterpene alcohols. The analysis of the blue fractions so obtained resulted in the conception of azulene as an oxygenated compound1).

The isolation of azulene was accomplished by Sherndal2) in 1915 when he made use of its capacity to react with sulphuric and phosphoric acids3). The addition compounds formed were insoluble in petroleum ether which made possible the removal of all traces of the volatile oil. The addition compounds were decomposed by the addition of water and the azulene extracted from the reaction mixture with ether.

This procedure could not be depended upon to yield an entirely pure product as other constituents of volatile oils often exhibit this property of adding mineral acids4), for example, cineol. Therefore, the preparation of a crystalline derivative was desirable. Since azulene will react with picric acid5) to yield an addition product which is easily hydrolized, this derivative, as well as the styphnate, was recommended by Ruzicka6) for the purpose of obtaining the pure hydrocarbon.

A method of isolation was also suggested by Herzenberg7) whereby a solid addition product was obtained by the addition of ferrocyanic acid to azulene.

The yield of azulene from various sources is shown in the following tabulation:

Source	percent yield	Investigator
Milfoil	1.6	Ruzicka and Rudolph[8)]
Chamomile	4.6	Ruzicka and Rudolph[9)]
Chamomile	1.4	Ruhemann and Lewy[10)]
E. globulus	2.4	Ruzicka and Rudolph[11)]
Guajene	20.0	Ruzicka and Rudolph[12)]
Artemisia Arborescens	50.0	Pellini and Morani[13)]
Gurjunene	4-5.0	Herzenberg and Ruhemann[14)]

Isolation of azulene from oil of Milfoil.

The material used consisted of ten lots of oil distilled from the flowering herb harvested in the garden of the Wisconsin Pharmaceutical Experiment Station under the direction of Professor W. O. Richtmann. The weight of green material, also the volume of the oil, together with the name of the distiller are herewith recorded.

Year	Herb	Orig.Oil	Cohob. oil	2nd. cohob.	Distiller
1924	1200 lbs.	720 cc.	250 cc.	--------	Bacon
1925	1600 "	2100 "	200 "	--------	Bacon
1926	3725 "	2655 "	255 "	80 cc	Christensen
1927*					
1928*					
1929	1000 "	550 "	65 "	20 "	Bienfang

* The crop of 1927 and 1928 was not distilled but was dried for Mr. MacMurry.

The oil was fractionated, the aldehydes removed and the esters saponified. It was steam distilled and again fractionated and the portion boiling above 200° (1460 cc) was taken for the isolation of azulene.

I. Fractionation of the oil.

The 1460 cc previously obtained were fractionated in 200 to 230 cc lots under 4 mm. pressure with the following results:

-80°	132 cc
80-85°	237 "
85-90°	107 "
90-100°	118 "
100+°	862 "
total	1456 cc.

All the fractions were deep blue in color. No constants were ascertained. From all of the fractions except the one obtained above 100°, a white crystalline solid separated at reduced temperature. These were separated from the oil on a force filter. The combined solids amounted to 200 gms. The liquid portions were again fractionated under 4 mm. pressure with the following results. Lots varying from 60 to 275 cc. were thus fractionated at a time.

-75°	15 cc.
75-80°	65 "
80-85°	100 "
85-90°	164 "
90 -95°	30 "
95-100°	47 "
100-110°	207 "
110-120°	207 "
120-130°	955 "
130-140°	84 "
140-160°	65 "
160-180°	45 "
180-200°	25 "
Residue	90 "

At 200° all of the blue oil had distilled over and the residue in the flask consisted of a yellowish- semi-solid mass.

The fractions boiling above 95° had a blue color and were used for the separation of azulene.

II. Isolation of azulene.

The separation of azulene from the fractions was attempted both by the sulphuric acid and phosphoric acid methods, with no apparent variation in the results. The phosphoric acid method was adopted for the bulk of the separations. The results are shown in the following tabulation:

Fraction		Reagent used	Products recovered		
B.p. at 4 mm	Amount		Oil	Non-vol. residue	Azulene
95-100°	23.5 cc	H_3PO_4	11.5 cc	4.5 gm	trace
95-100°	23.5 "	H_2SO_4	12.5 "	5 .0 "	trace
100-110°	30.0 "	H_3PO_4	17.5 "	11.0 "	1.0 cc.
100-110°	30.0 "	H_2SO_4	13.2 "	11.0 "	1.0 "
100-110°	30.0 "	H_3PO_4	17.7 "	10.0 "	1.0 "
100-110°	82.0 "	"	55.0 "	30.0 "	2.0 "
110-120°	100.0 "	"	80.0 "	15.0 "	1.8 "
120-130°	100.0 "	"	85.0 "	13.0 "	1.4 "
130-130°	95.0 "	"	61.0 "	19.0 "	4.1 "
130-140°	79.0 "	"	55.0 "	19.0 "	6.2 "
140-160°	58.0 "	"	31.0 "	12.0 "	11.0 "
160-180°	47.0 "	"	20.0 "	11.0 "	9.9 "
180-200°	16.0 "	"	6.0 "	5.0 "	1.7 "

III. Purification of Azulene. The picrate was prepared from this azulene in three lots.

Azulene used	Picrate	M.p.	Regenerated azulene
3.5 cc	3.0 gm.	113-115°	1.5 cc
13.0 "	11.0 "	113-115°	4.4 "
23.5 "	20.0 gm	113-114°	9.0 "

The regenerated azulene was distilled at 13.5 mm. pressure with the following results.

130-145°	1.0 cc
145-160°	1.1 "
160-165°	8.0 "
Residue abt.	4.0 "

This azulene was used in the feeding experiments (see therapeutic properties.)

1.) The early ideas of the composition of azulene are here with tabulated:

B.p. of fraction	Formula	Investigator and ref.
289-290°	$C_{20}H_{15}O$	Moessmer, Ann., 119 (1861) p. 262.
576° F.	$C_{16}H_{13}O$	Piesse, Ph. Journ., 23(1863) p. 277.
285-290°	$C_{20}H_{30}O$	Kopp, Arch.d.Ph., 209 (1876) p. 193.
165-170°(10mm)	$(C_{10}H_{17})_2O$	Barbier and Bouvealt, C.r., 119 (1894) p. 281.
300°	$(C_{15}H_{26}O)$	Alpers, Am. J. Ph., 71 (1899) p. 370.

2.) Sherndal, Journ. Am. Chem. Soc., 37 (1915) p. 167.

3.) This property of azulene had previously been noted by Moessmer, Kachler, and Kopp but this was apparently unknown to Sherndal who states "The property which was utilized to isolate the substance and which has heretofore not been noted, is its solubility in mineral acids of certain strengths."

4.) Kopp, Arch. d. Ph., 209 (1876) p. 193.

5.) Sherndal, Journ. Am. Chem. Soc., 37 (1915) p. 1537.

6.) Ruzicka and Rudolph, Helv. chim. acta, 9 (1926) p. 118.

7.) Herzenberg and Ruhemann, Ber., 58 (1925) p. 2258.

8.) Ruzicka and Rudolph, Helv. chim. acta, 9 (1926) p. 131.

9.)Ruzicka and Rudolph, Helv. chim. acta, 9 (1926| p. 132.

10.) Ruhemann and Lewy, Ber., 60 (1927) p. 2466.

11.) Ruzicka and Rudolph, Helv. chem. acta, 9 (1926) p. 133.

12.) Ruzicka and Rudolph, Helv. chem. acta, 9 (1926) p. 134.

13.) Pellini and Morani, Annali di Chimica Applicata, 7 (1923) p. 97; from Ber Schimmel and Co., (1924) p. 4.

14.) Herzenberg and Ruhemann, Ber., 58 (1925) p. 2259.

Artificial Preparation of azulene(s) Certain hydrocarbons, $C_{15}H_{24}$, yield blue products when oxidized. Thus, as early as 1887, it was observed by Wallach that cadinene in acetic acid solution when acted upon by a drop of sulphuric acid, yielded a blue color1). A like color reaction obtained by Wallach in 1887 with sylvestrene2), $C_{10}H_{16}$, is, in all probability, attributable to sesquiterpene impurities, for it is not likely that the reaction will effect a synthesis. Moreover, "pure" sylvestrene yielded but very little blue color when acted upon3). Oil of amyris, which appears to contain no sesquiterpene, but does contain a sesquiterpene alcohol, yields a blue color with this reagent4). Apparently the sulphuric acid first acts as a dehydrating agent. This deloration with sulphuric acid has been noted also with oil of guaiac4), oil of gurjun4), eucalyptus oil4), and caparrapene5). Inasmuch as a one percent solution of azulene in ether has a deep indigo blue color, it is apparent that the production of but infinitessimal amounts of azulene suffice to yield the color reaction described, upon oxidation of $C_{15}H_{24}$ to $C_{15}H_{18}$, the sulphuric acid in all probability acting as the oxidizing agent.

$$C_{15}H_{24} + H_2SO_4 \longrightarrow C_{15}H_{18} + SO_2 + H_2O$$

Atractylol, another sesquiterpene alcohol, yields a blue color with potassium bisulphate, whereas guajol does not6).

A similar color reaction has been reported when bromine is allowed to act on aromadendrene7), vetivene8), ledene9), guajene and eudesmene10). This reaction may be explained by substitution and subsequent splitting off of hydrogen bromide.

$$C_{15}H_{24} \longrightarrow C_{15}H_{21}Br_3 \longrightarrow C_{15}H_{18}$$

Sulphur also effects dehydrogenation as shown by Ruzicka in connection with guajene11) and the sesquiterpene fraction of eucalyptus oil. It has also been used to dehydrogenate cadinene12), oil of gurjun, glubulol, the sesquiterpene fraction of hyssop oil13) and aromadendrene.14)

Other agents which have been used are: zinc chloride and phosphorus with guajol15), palladium carbon with kessyl alcohol16), also nickel with cadinene and gurjun balsam oil17).

Finally heat alone seems to effect dehydrogenation as demonstrated by Semmler and Jakubwicz in connection with α-gurjunene18). This may also explain the presence of a blue fraction, yielding a picrate, m.p. 117°, in oils from the tar of brown coal19). Dry distillation of resin of Ferula Opopanax, Nardostachys Jatamansi20) and galbanum21) yields a blue oil. A blue oil has also been obtained by the dry distillation of calcium adipate 22) and by the condensation of acetylene over copper23).

For the purpose of affording a more ready oversight, the several reactions are herewith tabulated.

At room temperature

Sulphuric acid	Bromine
Guaiac oil4)	Aromadendrene7)
Caparrapene5)	Vetivine8)
Eucalyptus oil4)	Ledene9)
Gurjun oil4)	Guajene10)
Amyris oil4)	Eudesmene10)
Rosewood oil24)	Rosewood oil24)
Carotin25)	Carotin25)

At higher temperatures

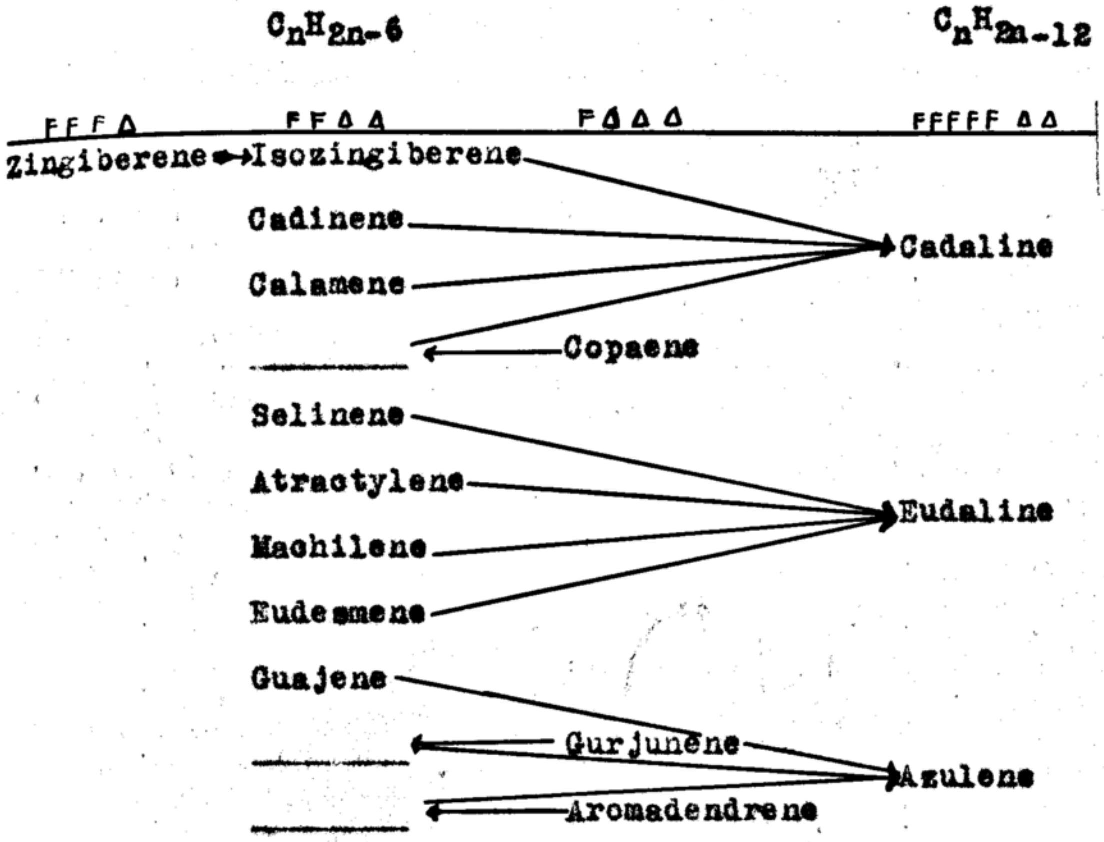
Heat	Sulphur	Palladium carbon	Nickel	Other agents
Calcium adipate22)	Guajene11)	Kessyl alcohol16)	Gurjun oil17)	Guajol15)
Resin of galbanum21)	Euc. Glob. oil11)			
Resin of Nard. Jat.20)	Gurjun oil13)			
α-gurjunene18)	Hyssop oil13)			
Oil of brown coal tar.19)	Aromadendrene14)			

The dehydrogenation of sesquiterpenes affords a means of insight into their structure. Dehydrogenation of a number of sesquiterpenes and sesquiterpene fractions has yielded evidence that their structures are similar since the dehydrogenation products generally belong to one of two or three types.

The dehydrogenation products usually obtained are cadaline, $C_{15}H_{18}$, eudaline, $C_{14}H_{16}$, and azulene, $C_{15}H_{18}$. Ruzicka assumes that the first two compounds result upon the dehydrogenation of bicyclic sesquiterpenes. Therefore the formation of cadaline from zingiberene, a mono-cyclic sesquiterpene, requires a rearrangement to a bicyclic form such as isozingiberene. Copaene, a tricyclic sesquiterpene, may yield the bicyclic cadaline because of an unstable three membered ring which readily allows the rearrangement to a bicyclic structure. A similar assumption may account for the formation of azulene from tricyclic sesquiterpenes.

Since the structure of cadaline and eudaline is known, the structure of the parent sesquiterpenes becomes correspondingly clearer. A knowledge of the structure of azulene will also make possible a further advance in the chemistry of the sesquiterpenes.

This relationship of the sesquiterpenes is made more evident in the following table.



Experimental. The material used consisted of two pounds of oil of guaiac wood concrete obtained from Schimmel and Co.

I. Isolation of guajol. The crystals from 933 gms. of oil of guaiac wood concrete were washed with petroleum ether and the crystals separated on a force filter. After washing several times with petroleum ether, they were crystallized three times from alcohol, when they had a melting point of 89-90°. Yield, 61 gms. (6.5 per cent)

II. Preparation of guajene. 765 gms. of a viscid yellow oil remained. This oil was heated for one hour with an equal weight of formic acid. The formic acid layer was removed and the oil fractionated under reduced pressure.

B.p. at 10 mm	Amount	d_{25}	N_d	Sap.V.	after~~p~~acetyl
125-135°	163 gms	0.9175	1.5000	4.3	21.89
135-145°	296 "	0.9300	1.5020	22.7	39.8
145-155°	55 "	0.9640			
Residue	83 "				

III. Dehydrogenation with sulphur. The above fractions were dehydrogenated with sulphur using three atoms of sulphur for each molecule of sesquiterpene. The hydrogen sulphide evolved was collected in lead acetate solution and from the amount of lead sulphide formed, the progress of the dehydrogenation was followed. The results are shown in the following table.

Fraction 10 mm	Amount	P_2O_5	Oil recovered	Azulene*
125-135°	62 gms	---	51.5 gms	7 gms
125-135°	85 "	85 gms	35.0 "	13 "
135-145°	62 "	96 "	26.0 "	20 "
135-145°	62 "	108 "	23.0 "	11 "
135-145°	62 "	91 "	18.0 "	12 "
135-145°	51.5 "	86 "	20.0 "	8 "
145-155°	52.5 "	127 "	16.0 "	8 "

From the azulene thus obtained the picrate was prepared, which after crystallization from alcohol melted at 115°. Yield, 25 gms. The picrate was decomposed with with sodium hydroxide and the azulene extracted with ether, and distilled at 50 mm pressure. 6 gms of azulene boiling at 201° were obtained leaving a non-volatile residue of 6 gms.

* regenerated from the phosphate.

1.) Wallach, Ann., 238 (1887) p. 87.

2.) Wallach, Ann., 239 (1887) p. 27.

3.) Herzenberg and Ruhemann, Ber., 58 (1925) p. 2261.

4.) Sherndal, J. Am. Chem. Soc., 37 (1915) pp. 168 and 1539.

5.) Tapia, Bull. Soc. Chem., 19, iii, 1638; Parry, Essential Oils, p. 93.

6.) Gadamer and Amenomiya, Arch. d. Ph., 241 (1903) p. 22.

7.) Smith, Journ. Roy. Soc. N. S. W., 35 (1901) p. 124.

8.) Langlois and Genoresoe, C.r., 135 (1903) p. 1059.

9.) Rizza, Journ. Russ. Phy. Chem. Soc., 19 (1887) p. 319; from Semmler, Die Aether. Oele, II, p. 539.

10.) Briggs and Short, J. Chem. Soc., (1928) p. 2525.

11.) Ruzicka and Rudolph, Helv. Chim acta, 9 (1926) p. 134.

12.) Ruzicka and Meyer, Helv. Chim. acta, 4 (1921) p. 508.

13.) Ruzicka, Pontalti and Balas, Helv. Chim. acta, 6 (1923) p. 857.

14.) Briggs and Short, J. Chem. Soc., (1928) p. 2526.

15.) Wallach and Tuttle, Ann., 279 (1894) p. 396.

16.) Asahina and Nakanishi, J. Ph. Soc. Japan, 48 (1928) p. 1.

17.) Herzenberg and Ruhemann, Ber., 58 (1925) p. 2258.

18.) Semmler and Jakubwicz, Ber., 47 (1914) p. 2256.

19.) Ruhemann and Lewy, Ber., 60 (1928) p. 2459.

20.) Semmler, Die Aether. Oele, III, p. 263.

21.) Moessmer, Ann., 119 (1861) p. 262.

22.) Hentschel and Wislicenus, Ann., 275 (1893) p. 312.

23.) Schaeffer and Stadler, Helv. Chim. acta, 9 (1926) p. 193.

24.) Penfold, J. Proc. Roy. Soc., N.S.W., 61 (1927) p. 337.

25.) Kuhn, Winterstein and Wiegand, Helv. Chem.acta, 11,(1928) p. 716.

Physical Properties. Only those constants recorded for regenerated azulene are worth considering. (See isolation) At room temperature azulene is a liquid. In bulk it appears almost black, but is intensely blue in thin layers.1) A one per cent solution in ether is intensely blue in color.

Its odor is described as weakly phenolic, suggesting thymol, especially when warm.1)

Boiling point:- Under atomospheric pressure it was found to boil between 295 and 300° leaving a tarry residue. At 25 mm. pressure it boiled at 185-195° leaving a brown residue of about 5 per cent2). Under a pressure of 11 mm. it boiled at 167-168° with a clear cut spearation from the residue3). Other boiling points which have been reported are as follows:

Temp.	pressure	source	investigator
135.0°	0.5 mm	E. Glob.	Ruzicka4)
135.6°	1.1 "	Milfoil	R. Kremers5)
153.0°	7.0 "	Kessyl alcohol	Asahina and Nakanishi6)
158-161°	11.0 "	Chamomile	Ruzicka4)
164°	11.0 "	Guajol	Ruzicka4)
167-168°	11.0 "	Milfoil	R. Kremers5)
161°	12.0 "	Milfoil	Ruzicka4)
163°	14.0 "	Chamomile	Ruhemann and Lewy7)

Specific gravity:- Though prepared from different sources the density of the regenerated hydrocarbon has been found to vary but little.

d	t	source	regenerated from	Author
o.9728	25°	-------------	Phosphate	Sherndal
0.9846	25°	milfoil		R.Kremers
0.9883	20°	milfoil	Picrate	Ruzicka
0.9881	18°	chamomile	Picrate	Ruzicka
0.9759	18°	guajol	Picrate	Ruzicka
0.9717	24°	Kessyl alcohol	Picrate	Asahina and Nakanishi

Solubility:- Azulene is readily soluble in most organic solvents. It is miscible with 95 per cent alcohol in all proportions. 50 per cent alcohol dissolves enough to impart a decidedly blue tint to the solution. It is soluble in petroleum ether, ether, acetone, chloroform, carbon tetrachloride, carbon disulphide, heptane, and ethyl acetate.

It is soluble in strong sulphuric, phosphoric and hydrochloric acids, from the solutions of which it is precipitated upon the addition of water. It behaves simularily with nitric acid at reduced temperature but at room temperature, oxidation occurs. (see chemical properties)

Azulene is soluble in formic acid from which it can be regenerated by the addition of water. It is not soluble in other organic acids.

The Index of Refraction and Optical Rotation have not been determined because of its intense color.

Absorption spectrum:- The absorption spectrum of the blue oils received considerable attention from early investigators8) who sought to establish the identity of the blue substances in the different oils.

R. Kremers[9)] made a thorough investigation of the absorption bands of the regenerated hydrocarbon. The measurement of these bands has been used by Herzenberg and Ruhemann[10)], Ruzicka and Rudolph[11)], and Asahina and Nakanishi[12)] to indicate a similiarity between the azulenes from various sources.

Ruzicka and Rudolph have also investigated the spectrum of octohydroazulene and found that here the characteristic absorption is only in the outermost ultraviolet. From this they conclude that the absorption bands of azulene in the visible spectra are produced by the position of the double bonds and that bands due to the hydrocarbon structure will be found in the ultra violet.

1.) Sherndal, J. Am. Chem. Soc., 37 (1915) p. 169.

2.) Sherndal, J. Am. Chem. Soc., 37 (1915) p. 170.

3.) R. Kremers, J. Am. Chem. Soc., 45, (1923) p. 718.

4.) Ruzicka and Rudolph, Helv. Chim. acta, 9 (1926) pp. 132, 133 and 134.

5.) R. Kremers, J. A. Ph. A., 10, (1921) p. 258.

6.) Asahina and Nakanishi, J. Ph. Soc. Japan, 48 (1928) p. 1.

7.) Ruhemann and Lewy, Ber., 60 (1927) p. 2466.

8.) Brewster, David, Ph. Journ., 23 (1863) p. 2466.
Gladstone, J. Chem. Soc., 17 (1864) p. 1.
Tschirch, Arch d. Ph., 223 (1895) p. 278.
Hook, Arch. d. Ph., 221 (1883) p. 17.

9.) R. Kremers, J. Am. Chem. Soc., 45 (1923) p. 717.

10.) Herzenberg and Ruhemann, Ber., 58 (1925) p. 2249.

11.) Ruzicka and Rudolph, Helv. Chim. acta, 9 (1926) p. 118.

12.) Asahina and Nakanishi, J. Ph. Soc. Japan, 48,(1928) p. 1.

Chemical Properties. Because of its highly unsaturated character, the additive reactions of azulene have attracted special attention.

Addition of hydrogen. The reduction of azulene has been studied by Sherndal1)(1915), Augsburger2)(1915), R. Kremers3) (1923), Ruzicka4)(1926), Ruhemann5)(1927) and Asahina and Nakanishi6)(1928). There is a difference of opinion as to whether eight or ten hydrogen atoms are added. For the convenience of ready comparison, the results are herewith tabulated:

Azulene used				Reduction Product			
Source	B.p.	d.	Formula	B.p.	d	Ref. Index	Mol. Refr.
-----1)	295-300°	0.9738	$C_{15}H_{26}$		0.8935	1.4900	67.30
Milfoil2)	163° at at 12 mm.		$C_{15}H_{28}$	115-116° at 12 mm			
Milfoil3)	167-168.4° at 11 mm.	0.9846	$C_{15}H_{28}$	130-140° at 20 mm	0.8920	1.4870	67.06
Milfoil4)	161° at 12 mm.	0.9883	$C_{15}H_{26}$	119° at 11 mm	0.8932	1.4856	66.16
Guajol4)	164° at 11 mm.	0.9759	$C_{15}H_{26}$	123-125° at 11 mm	0.8872	1.4834	66.38
E. Glob.4)	164° at 11 mm.	0.9757					
Chamomile4)	159° at 11 mm.	0.9881					
Chamomile5)	163° at 11 mm.		$C_{15}H_{26}$	123-124° at 10 mm	0.8967	1.4921	
Kessyl6) alcohol	153° at 7 mm	0.9717	$C_{15}H_{26}$	112-115° at 7.5mm	0.8930	1.4714	64.52

Other reductions of azulene have also been studied. Sherndal attempted to reduce the hydrocarbon with sodium and alcohol but without success. R. Kremers also failed to obtain reduction by this means. Ruzicka, however, succeeded in reducing the hydrocarbon using sodium and amyl alcohol, obtaining thereby a product boiling at 118-124° at 11 mm. The analysis of this product indicated the formula $C_{15}H_{24}$.

R. Kremers found[7)] that azulene could not be reduced with alumnium amalgam.

Addition of halogen:- Azulene readily enters into reaction with the halogens. With bromine in petroleum ether solution a lemon yellow precipitate is formed, which immediately begins to regenerate the blue color. Similar results are obtained with iodine.

When chlorine is passed into a petroleum ether solution of azulene the solution is decolorized with the formation of a sticky, black precipitate. Upon the further addition of chlorine, this precipitate assumes an orange color, with the evolution of hydrogen chloride.

Addition of thiocyanogen: Azulene also enters into reaction with the halogen-like group, thiocyanogen, forming in acetic acid solution, a dark solution from which azulene is regenerated by the addition of water.

Addition of hydrohalogen:- Sherndal states that azulene apparently does not react with hydrochloric acid. However, it has been found that hydrochloric acid behaves in a manner similar to the other mineral acids, that is, the azulene forms a reddish liquid compound from which azulene can be again regenerated by the addition of water. Gaseous hydrogen chloride, when passed into an ethereal solution of azulene, forms a white compound which immediately begins to regenerate the blue color.

Hydrobromic and hydriodic acids also decolorize azulene forming similar unstable compounds.

Addition of oxygen:- The oxidation of azulene with potassium permanganate was attempted by Sherndal who obtained only lower fatty acids. R. Kremers, using Wallach's alkaline permanganate method, got very low yields. He identified acetic acid and acetone in the oxidation mixture and obtained a solid acid in traces, which on the basis of a color reaction, seemed to be a methyl phthalic acid.

Ruzicka repeated this oxidation and also obtained traces of a substance comparable to the methyl phthalic acid of kremers. Ruzicka however, was unable to find acetone in the reaction mixture.

Asahina and Nakanishik by the ozonization of kessazulene have identified formic and acetic acids, acetone and oxalic acid as the "abbau" products of azulene.8) They also reported the absence of acids higher than propionic acid.

Oxidation with nitric acid:- 1 gm. of azulene was added, drop by drop to 3 cc of concentrated nitric acid cooled to 0°. A reddish-brown oil separated and was removed. When washed with water, it solidified, yielding 1.4 gm. of a dark brown, amorphous solid. The nitric acid solution yielded no further product when diluted with water.

Oxidation with benzoyl hydroperoxide:- 1.8 gms. of azulene were dissolved in 20 cc of chloroform and added drop by drop to ten per cent excess of benzoylhydroperoxide in chloroform solution. The addition required about 20 minutes and the reaction mixture was maintained at 0°. The azulene was instantly decolorized. The chloroform was removed by distillation and the residue extracted with dilute sodium hydroxide solution to remove benzoic acid. The resulting brown, viscid oil was shaken with 1 per cent sulphuric acid for three days. The resulting oil (0.5 gm) was not further examined.

Addition of nitrogen groups:- Azulene adds to nitric acid in concentrated solution when cooled to 10°. From this compound azulene can be regenerated with water. If, however, the reaction mixture reaches room temperature, oxidation takes place, yielding the amorphous yellow substance described by Sherndal (See oxidation)

Azulene readily reacts with nitrogen trioxide and nitrosyl chloride but no characteristic product has been isolated.

Addition of picric acid and trinitroresorcin:- The addition of picric acid and of trinitroresorcin has afforded a convenient method of purification of azulene. (see isolation) Picric acid yields jet black, shiny needles, which can be recrystallized from alcohol. Ether, carbon tetrachloride, and benzene break up the compound dissolving out the azulene. The azulene may also be liberated by the addition of water. A quantitative determination of the picric acid shows that one mol of picric acid combines with one mol of the hydrocarbon9)

For comparison the melting points of the various picrates and styphnates are tabulated:

source	Picrate	Styphnate	Investigator
Cubeb oil	122°		Sherndal
Camphor oil	118°		Sherndal
Gurjun oil	122°		Sherndal
Milfoil	110-115°		R. Kremers
Gurjun oil	116-117°		Herzenberg and Ruhemann
Generator tar oil	116°		Herzenberg and Ruhemann
Chamomile oil	118°		Ruzicka and Pontalti
Milfoil oil	115°	95-96°	Ruzicka and Rudolph
Chamomile oil	115°	92-93°	Ruzicka and Rudolph
E. Globulus oil	118-120°	122-123°	Ruzicka and Rudolph
Guajol	122°	105-106°	Ruzicka and Rudolph
Kessyl alcohol	123°	106°	Asahina and Nakanishi

Other addition reactions: The addition of sulphuric acid and phosphoric acid to azulene have already been discussed. (see isolation). The addition of ferrocyanic acid to azulene yields a white crystalline compound[10] whose analysis indicates that one mol of ferrocyanic acid adds to one mol of azulene.

Other reactions of azulene:- Strong alkalis, even with heat, do not affect the color of azulene. It also cannot be isomerized with acids[11].

By the action of acetic anhydride and sulphuric acid, a substance has been obtained which is apparently a sulphonic derivative[12].

The decolorization of azulene by metallic sodium was pointed out by early investigators as evidence of an oxygenated structure. The discovery of its hydrocarbon nature left this reaction unexplained. It was found by R. Kremers that the addition of water would again precipitate the azulene.

1.) Sherndal, J. Am. Chem. Soc., 37 (1915) p. 1542.

2.) Augsburger, Science (New Series) 42 (1915) p. 100. Also unpublished data and letters sent to Dr. Kremers.

3.) R. Kremers, J. Am. Chem. Soc., 45 (1923) p. 720.

4.) Ruzicka and Rudolph, Helv. Chim. acta, 9 (1926) p. 118.

5.) Ruhemann and Lewy, Ber., 60 (1927) p. 2459.

6.) Asahina and Nakanishi, J. Ph. Soc. Japan, 48 (1928) p. 1.

7.) R. Kremers, J. Am. Chem. Soc., 45 (1923) p. 720.

8.) Asahina and Nakanishi, J. Ph. Soc. Japan, 52 (1932) p. 2.

9.) Sherndal, J. Am. Chem. Soc., 37 (1915) p. 1538.

10.) Herzenberg and Ruhemann, Ber., 58 (1925) p. 2258.

11.) Ruzicka and Rudolph, Helv. Chim. acta, 9 (1926) p. 118.

12.) Sherndal, J. Am. Chem. Soc., 37 (1915) p. 170.

Therapeutic properties: The therapeutic properties of azulene have received slight consideration, due largely to the general idea that hydrocarbons of high molecular weight possess little physiological activity. The study of the hydrocarbons has, however, been stimulated by the discovery of the relationship of carotin to the vitamins. The question arises whether azulene, also a highly colored, unsaturated hydrocarbon, may not be similarly related. The fact that investigators working with this compound have noticed an increased appetite lends weight to the supposition that it may in some way stimulate metabolism.

Loevenhart's[1)] discovery that it possessed toxic action was also surprising and made a study of its therapeutic properties seem desirable.

At the same time, a study of substances related to azulene, namely the sesquiterpene alcohols, might also yield results of interest. For this purpose, guajol which yields azulene by dehydrogenation, was chosen.

Experimental* In the following investigation the azulene used was obtained from oil of milfoil by extraction with phosphoric acid and purified by regeneration from the picrate and subsequent distillation under reduced pressure. The irradiated azulene was prepared by exposing the alcoholic solution for thirty minutes in an atmosphere of nitrogen to the rays of an ultra-violet lamp placed at 18 inches distance. No change in color was apparent. The alcohol was removed by vacuum distillation and the irradiated azulene dissolved in wesson oil.

All solutions were made in wesson oil and were fed by mouth with a medicine dropper. All control animals received wesson oil.

The guajol used was obtained from oil of guaiac wood concrete by washing it with petroleum ether until the crystals obtained melted at 91°. It was irradiated by exposing the finely powdered crystals to ultra-violet light, placed at 18 inches for 30 minutes and a solution prepared by warming the alcohol with wesson oil at a temperature of aproximately 50°.

In all experiments rats were used and kept in separate cages on screens. Daily consumption records were kept.

<u>Effect on appetite</u>. Six animals weighing between 60 and 70 gms. were placed on a normal diet[2]. In addition,

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* The assistance of Professor Steenbock, who directed these experiments is gratefully acknowledged. Thanks are also due to Miss Riising and Miss Van Donk for their co-operation and assistance in the laboratory.

three of these animals were given one drop of a solution of azulene containing 1 mg of azulene in each drop and three control animals were given 1 drop of wesson oil per day. This diet was fed for three weeks.

Then, while still continuing the basic diet, two of the animals received 1 drop of a solution containing 10 mg. of azulene per drop and one was given one drop (25 mg) of pure azulene. This diet was fed for ten days.

The average consumption records show that the consumption of the rats given azulene did not differ from that of the rats given only wesson oil. Therefore, it may be concluded that azulene has no effect on the appetite of rats.

Average consumption record for 21 days

Azulene per day	Rat	Consumption	Wesson oil	Rat	Consumption
1 mg.	1	11.0 gm.	20 mg.	4	11.1 gm.
1 "	2	10.6 "	20 "	5	11.0 "
1 "	3	10.0 "	20 "	6	10.7 "
average		10.5 gms	average		10.9 gms

Average consumption record for 10 days

Azulene per day	Rat	Consumption	Wesson oil	Rat	Consumption
25 mg.	1	12.6 gms.	20 mg.	4	10.9 gms
10 mg.	2	13.6 "	20 "	5	14.4 "
10 mg.	3	12.4 "	20 "	6	12.1 "
average		12.9 gms.	average		12.7 gms

<u>Effect of azulene on xeropthalmia:-</u> Three animals which had been fed on a diet deficient in vitamin A[3)] for six weeks and exhibited symptoms of xerophthalmia, were used for this experiment. Two rats were given each day 1 drop of a solution containing 1 mg. of azulene per drop and one rat was given 1 drop of cod liver oil.

The rats receiving azulene gradually lost weight and died on the 8th and 22nd day. The rat receiving cod liver oil increased in weight and was completely recovered on the 20th day.

<u>Effect of azulene on polyneuritis.</u>:- Seven animals which had been fed a diet deficient in vitamin B[4)] for seven weeks were continued upon that diet and in addition three of the rats received 1 mg. of azulene and four of the rats received 1 drop of wesson oil.

The four rats receiving wesson oil died within 5 to 14 days and those receiving azulene died within 5 to 8 days.

<u>Effect of azulene on rickets</u>:- Twelve rats were fed on a diet deficient in vitamin D[5)] for three weeks. When they were all rachitic, as evidenced by the peculiar gait and enlarged wrists, the diet of the various rats was supplemented daily by one of the following substances:

a.) 1 mg. of azulene
b.) 0.6 mg. of irradiated azulene
c.) 20 mg. of cod liver oil
d.) 20 mg. of wesson oil

After ten days the animals were killed, the wrists removed and examined for calcium deposition by staining a section of the wrist with silver nitrate solution.

In animals fed on azulene, irradiated azulene or wesson oil, there was no calcium deposition. Those fed on cod liver oil showed a medium line of calcium deposition in the wrists.

Effect of azulene on animals receiving a diet deficient in vitamin E.

Three male and three female animals were fed on a diet deficient in vitamin E6) for seven weeks. Two females fed on the same diet for ten weeks were also used.

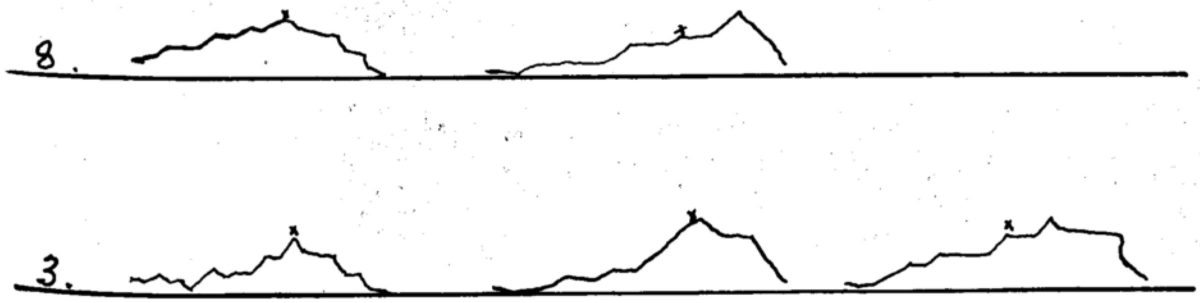
When the females weighed between 190 and 200 gms., they were mated and, by means of daily weight records, reabsorption curves for this period were plotted.

After reabsorption had taken place the females were given 1 mg. of azulene daily and again mated. Reabsorption curves were obtained as before.

Three of the females did not mate the second time and consequently no reabsorption curves could be obtained.

Reabsorption Curves

Rat Without azulene With azulene With azulene



* Red blood cells.

Effect of guajol on xerophthalmia. Four animals which had been fed on a diet deficient in vitamin A[3] for six weeks were then fed, in addition 1 drop of a solution containing 1 mg of guajol. The rats gradually lost weight and died after 9, 10, 17, and 28 days.

Effect of guajol on rickets. Six rats were fed on a diet deficient in vitamin D[5] until all showed the enlarged wrists and peculiar gait characteristic of rachitic animals.

Three of the rats then received 1 mg of guajol and three received 1 mg of irradiated guajol in wesson oil solution.

After ten days the animals were killed and the wrists examined by staining a section with silver nitrate solution. In no case was there evidence of calcium deposition.

1.) Journ. Pharmacol. and Exp. Therap., 17 (1921) p. 346.

2.)	Yellow corn	71.5 gm	2/3
	Alfalfa	2.0 "	
	Oil meal	15.0 "	
	Crude casein	5.0 "	
	Salt	0.5 "	
	Ca. acid phos.	1.0 "	
	Butter fat	5.0 "	
	Wheat germ oil	5 % of the butter fat	
	Whole milk powder		1/3

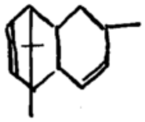
3.)	Casein 27 (alc. ext'd and heated)	180 gms
	Agar	20 "
	Yeast, non-irrad.	69 "
	Yeast, irrad.	1 "
	Salts,40	40 "
	Dextrin	690 "

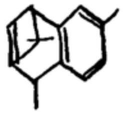
4.)	Casein 22(acetic acid ext.)	18 gms
	Yeast 40	1 "
	Salts 40	4 "
	Cod liver oil	2 "
	Dextrin	75 "

5.)	Yellow corn	76 gms
	Wheat gluten	20 "
	Calcium carbonate	3 "
	Sodium chloride	1 "

6.)	Casein 27(alc. ext'd and heated)	180 gms
	Agar	20 "
	Yeast, non irrad.	80 "
	Salts 40	40 "
	Cod liver oil	20 "
	Lard	220 "
	Dextrin	440 "

Structure. When the oxygenated conception of azulene was overthrown by Sherndal's isolation and analysis of the compound, its emperical formula was found to be $C_{15}H_{18}$, thereby placing it under the formula of saturation C_nH_{2n-12}. Since under this formula of saturation numerous configurations are possible, the problem became one of determining the number of cycles. As the refractive index cannot be determined, reduction is the only means of ascertaining this fact. Although the results are still in dispute, it is an accepted fact that azulene is a bicyclic compound with five double bonds , thereby eliminating the tricyclic formulas proposed by Sherndal.

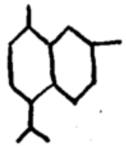




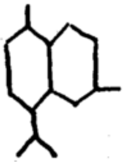
Calculated in accordance with the isoprene theory for the formation of terpenes and sesquiterpenes, there are possible for azulene the following types of ring structure with five and six membered cycles:

Six membered rings

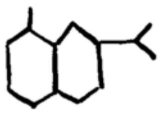
Type I



Type II

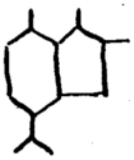


Type III

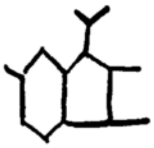


Five and six membered rings

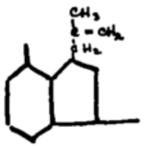
Type IV



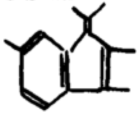
Type V

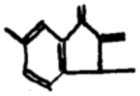


Type VI



The following formulas of type V have been proposed by R. Kremers since it is possible to harmonize the experimental facts with such a structure and possibly to account for the color of the molecule in the five membered ring.





Objection has been raised to this formula since the alkylated benzo-fulvenes do not show such strong color as azulene does. Ruzicka believes the color formation to be due solely to the peculiar arrangement of the double bonds since the absorption spectrum of hydrogenated azulene does not show any of the characteristic bands of azulene.

Also, the assumption of an aromatic ring in azulene, which is strongly indicated by the formation of a picrate which will regenerate the hydrocarbon, is questioned by Ruzicka.

Summary. The blue color of volatile oils has received considerable attention from early investigators. However, their investigations yielded such varying results, due to the impurity of the material with which they worked, that little of scientific value was derived from their efforts. The isolation of the bearer of the blue color by Sherndal in 1915 established its identity as a hydrocarbon, $C_{15}H_{18}$. Its structure has since been sought by recent investigators through its reduction and oxidation products, as well as through the absorption spectrum. These researches have shown azulene to be a bicyclic compound with five double bonds. Oxidation, however, has not given satisfactory results and it appears that these methods must be applied to the more stable reduction products rather than to the azulene itself in order to obtain satisfactory yields.

The importance of azulene to the sesquiterpenes is evidenced by color reactions of a large number of sesquiterpenes or sesquiterpene fractions. These sesquiterpenes, when treated with certain oxidizing agents yield a blue color, which in some cases has been identified as azulene. A knowledge of the structure of azulene may be expected to do much toward solving the structure of that group of sesquiterpenes. This artificial preparation is also of value to the research on azulene in providing a cheaper and more abundant source than the natural oils afford.

The variety of sources of azulene has raised a question as to the desirability of terming all blue substances azulene. The blue hydrocarbon, $C_{14}H_{16}$, obtained by Schlaepfer and Stadler points to a necessity of caution in the consideration of blue products. The melting points of the picrates and styphnates have yielded some evidence that the hydrocarbon material, $C_{15}H_{18}$, is different when prepared from different sources. This has resulted in the use of such names as guajazulene, chamazulene and kessazulene, denoting botanical source.

The wide spread occurence of azulene, indicated by the color of a large number of volatile oils, is probably significant of its biogenetic importance. The form of its existence in the plant has been the subject of much speculation and the theory of Herzenberg and Ruhemann, indicating a direct formation from the sesquiterpenes, is not supported by their own experiments and is entirely disproven in this investigation so far as milfoil in concerned. In this investigation an attempt was made to account for the presence of azulene in a form which cannot be extracted by petroleum ether, by the assumptäon of an acid salt addition product or by a glucosidal combination. The experimental data obtained, however, have not supported these theories. The azulene-bearing compound has been extracted and found to be a hydroxylated compound with an indication of an ester or lactone linkage. Both hydrolysis and oxidation yield only amorphous products, from which no characteristic derivatives have been obtained.

A study of the therapeutic properties of azulene, which have been neglected because of its hydrocarbon nature, has been rendered desirable by the recent discovery of the relationship of carotin to the vitamins. Azulene was found to be without effect in cases of a deficiency of vitamins A, B, D, and E, and to be without effect on the appetite of rats. However, the long established use of milfoil by the physicians and common people as a tonic is indicative of a therapeutic value, which if not in the azulene as such, may be present in the azulene compound as it occurs in the plant.