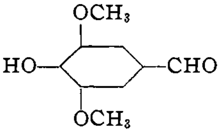
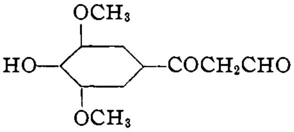
[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, McGILL UNIVERSITY]

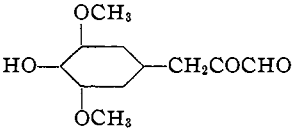
Studies on Lignin and Related Compounds. XLVIII. Identification of Vanillin and Vanilloyl Methyl Ketone as Ethanolysis Products from Spruce Wood[1]

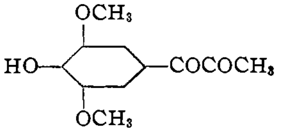
BY LEO BRICKMAN, W. LINCOLN HAWKINS AND HAROLD HIBBERT

In a recent communication[2] an investigation of the bisulfite-soluble fraction, obtained on ethanolysis of maple wood, was described. It was established at that time that this fraction consisted of a mixture of guaiacyl and syringyl components in approximately equal amounts. A comprehensive study of the syringyl components disclosed the presence of syringaldehyde (I) and a dicarbonyl compound of empirical formula $C_{11}H_{12}O_5$. This latter component contained the syringyl nucleus as shown by mild oxidation with hydrogen peroxide which resulted in formation of syringic acid in 65% yield, while preparation of a disemicarbazone indicated the presence of two carbonyl groups in the molecule. On the basis of this evidence, three possible structures for this product were suggested (II, III, IV).


(I)

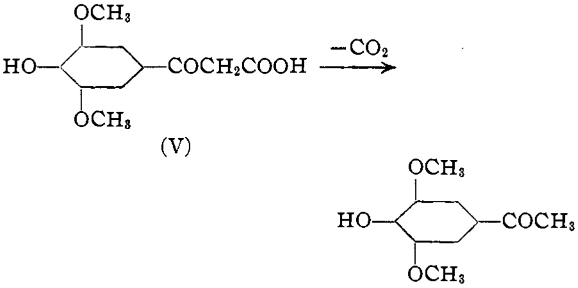

(II)


(III)


(IV)

Syringoylacetaldehyde (II) was believed, at that time, to be the most probable of these isomeric structures. Formula (III) was eliminated since hydrogen peroxide oxidation resulted in formation of 65% of syringic acid, and, assuming this structure, the reaction would proceed through syringaldehyde as one intermediate. It was conclusively shown, by control experiments, that syringaldehyde is stable under these conditions.

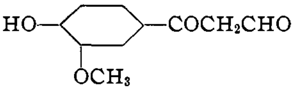
A decision between (II) and (IV) was then attempted on the basis of the following evidence: (a) The dicarbonyl compound apparently did not react with *o*-phenylenediamine. The conditions employed were those under which benzil reacts readily. (b) A negative iodoform test was obtained. (c) In one isolated experiment, when the dicarbonyl compound was subjected to the action of aqueous bisulfites, followed by alkaline cleavage, there was obtained acetosyringone. Presumably oxidation to the keto-acid (V) took place followed by decarboxylation to acetosyringone (VI). This result could not be duplicated.

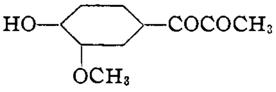

(V)

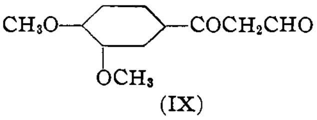
(VI)

These observations seemed to point to the structure of the dicarbonyl derivative as syringoylacetaldehyde (II). It was realized, however, that, until comparison of the natural product with synthetic syringoylacetaldehyde could be effected, this conclusion could not be regarded as definite and final.

Attention has recently been directed to a study of the bisulfite-soluble fraction of the ethanolysis products from spruce wood. Two components have been isolated, namely, vanillin and a dicarbonyl derivative, $C_{10}H_{10}O_4$, analogous to the assumed syringoylacetaldehyde. In this respect, the bisulfite-soluble fraction from spruce corresponds with the guaiacyl components from maple wood. In the case of the guaiacyl dicarbonyl derivative, however, synthesis of the structural possibilities, namely, vanilloylacetaldehyde (VII) and vanilloyl methyl ketone (VIII), could be effected more readily due to the abundance of vanillin and acetovanillone as starting materials.


(VII)


(VIII)


(IX)

---

[1] From a thesis presented to the Faculty of Graduate Studies and Research, McGill University, by Leo Brickman, in May, 1940, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Pyle, Brickman and Hibbert, THIS JOURNAL, **61**, 2198 (1939).

Both vanilloyl methyl ketone and veratroylacetaldehyde (IX) have now been synthesized, and it may be stated without reservation that the guaiacyl dicarbonyl component is vanilloyl methyl ketone (VIII) rather than vanilloylacetaldehyde (VII), as previously assumed. This conclusion has been verified by comparison of the 2,4-dinitrophenylhydrazones, mono- and disemicarbazones of the naturally-occurring and synthetic vanilloyl methyl ketone. Derivatives of synthetic veratroylacetaldehyde, on the other hand, gave substantial depressions of the melting point when mixed with the methylated analogs of the ethanolysis diketone product.

A reinvestigation of the constitution of the syringyl dicarbonyl component is now in progress and it has been found that this product is syringoyl methyl ketone (IV), proof of which has been provided by comparison with the synthetic substance. A description of this work will appear in a subsequent communication.

The synthesis of vanilloylacetaldehyde presented many difficulties. Attempts first were made to synthesize it by the action of allylmagnesium bromide on vanillin, followed by oxidation of the resulting secondary alcohol group and subsequent ozonization of the ethylenic double bond

$$\mathrm{RCHO + CH_2{=}CHCH_2MgBr \longrightarrow RCHOHCH_2CH{=}CH_2 \xrightarrow{CrO_3} RCOCH_2CH{=}CH_2 \xrightarrow{O_3} RCOCH_2CHO}$$

$$(\mathrm{R} = \text{guaiacyl})$$

This synthesis failed in the first step when vanillin was employed, and in other cases where the phenolic hydroxyl group of vanillin was blocked with a benzoate, methyl or methoxymethyl ether group it could not be carried to completion.

A second method of approach was suggested by the possibility of brominating acetovanillone, converting this to the nitrile and then effecting a Stephen reduction[3] to give vanilloylacetaldehyde.

$$\mathrm{RCOCH_3 \xrightarrow{Br_2} RCOCH_2Br \xrightarrow{KCN} RCOCH_2CN \xrightarrow[SnCl_2]{[H]} RCOCH_2CH{=}NH{\cdot}HCl \xrightarrow{HOH} RCOCH_2CHO}$$

$$(\mathrm{R} = \text{guaiacyl})$$

In the case of acetovanillone acetate, this synthesis could be carried up to, but not beyond, the final reduction stage, all attempts to reduce the nitrile being unsuccessful.

Finally, the Claisen–Schmidt condensation[4] was employed and, although this reaction did not take place with either acetovanillone, its acetate or methoxymethyl ether, acetoveratrone reacted smoothly to give veratroylacetaldehyde in good yield.

$$\mathrm{RCOCH_3 + Na + HCOOEt \longrightarrow RCOCH{=}CHONa \xrightarrow{HOH} RCOCH_2CHO}$$

$$(\mathrm{R} = \text{veratryl})$$

This synthetic veratroylacetaldehyde formed a crystalline 2,4-dinitrophenylhydrazone which gave a marked depression of the melting point (19°) when mixed with the corresponding methylated derivative of the naturally-occurring product.

Vanilloyl methyl ketone (VIII) was prepared by mild oxidation of α-hydroxypropiovanillone (recently synthesized by Cramer and Hibbert[5]) using copper sulfate and pyridine as employed by Clarke and Dreger[6] in the oxidation of benzoin to benzil

$$\mathrm{RCOCHOHCH_3 \longrightarrow RCOCOCH_3}$$

The mono- and disemicarbazones and 2,4-dinitrophenylhydrazone of the synthetic product were found to be identical with the corresponding derivatives of the natural material, thus establishing the identity of the latter as vanilloyl methyl ketone.

Separation of the Carbonyl Constituents in the Bisulfite Ethanolysis Fraction.—After numerous attempts to find a method for separating vanil-

TABLE I

Ethanolysis experiment no.	1	2	3
Low-boiling fract. taken (fract. (a), Chart I), g.	0.09	0.14	0.18
Pptd. 2,4-dinitrophenylhydrazone, g.	0.16	0.18	0.31
Vanillin 2,4-dinitrophenylhydrazone, g.	0.04	0.0	0.05
Vanillin in low-boiling fract. (a), %[a]	20.3	0.0	12.7
2,4-Dinitrophenylhydrazone of vanilloyl methyl ketone, g.	0.06	0.07	0.15
Vanilloyl methyl ketone in low-boiling fract. (a), %	34.6	26.0	43.2
Vanillin and vanilloyl methyl ketone in low-boiling fract. (a), %	54.9	26.0	55.9

[a] It is possible that very large differences in the yield of vanillin are to be associated with the age of the tree and growth conditions such as climate and soil. These factors are to be investigated.

---

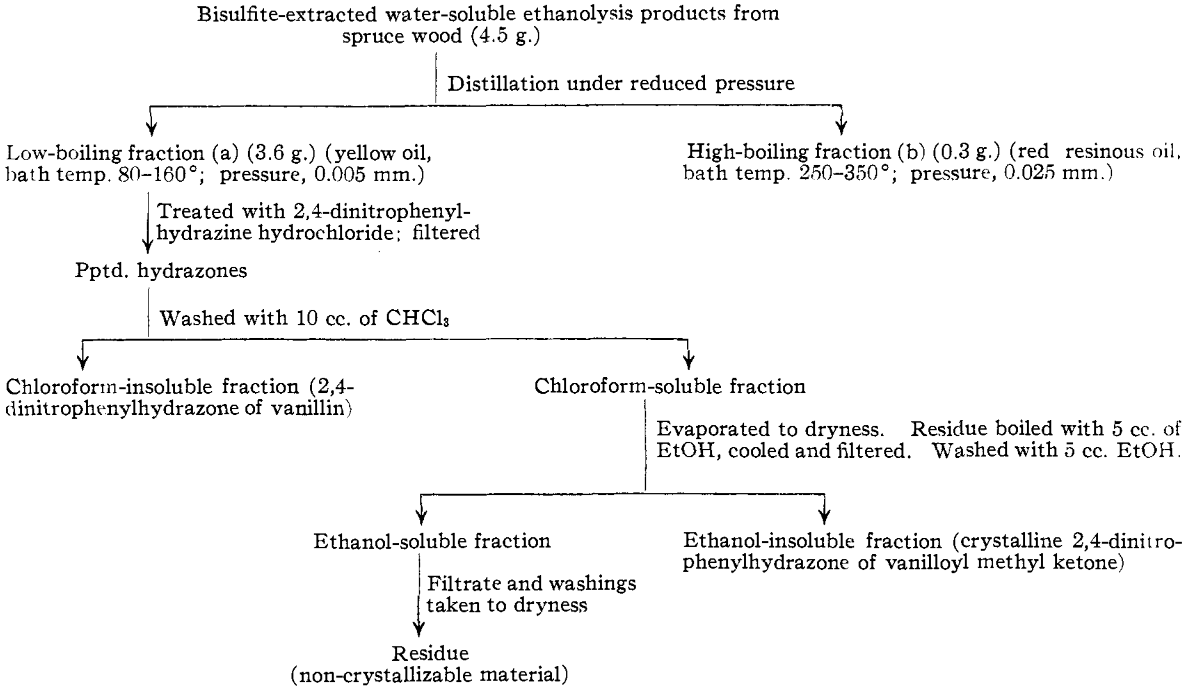
[3] (a) Stephen, *J. Chem. Soc.*, **127**, 1874 (1925); (b) McCord, THIS JOURNAL, **53**, 4181 (1931); (c) Law and Johnson, *ibid.*, **52**, 3627 (1930).

[4] (a) v. Auwers and Schmidt, *Ber.*, **58**, 528 (1925); (b) v. Auwers and Ottens, *ibid.*, **58**, 2072 (1925).

[5] Cramer and Hibbert, THIS JOURNAL, **61**, 2204 (1939).

[6] Clarke and Dreger, "Organic Syntheses," Vol. VI, 1926, p. 6.

CHART I

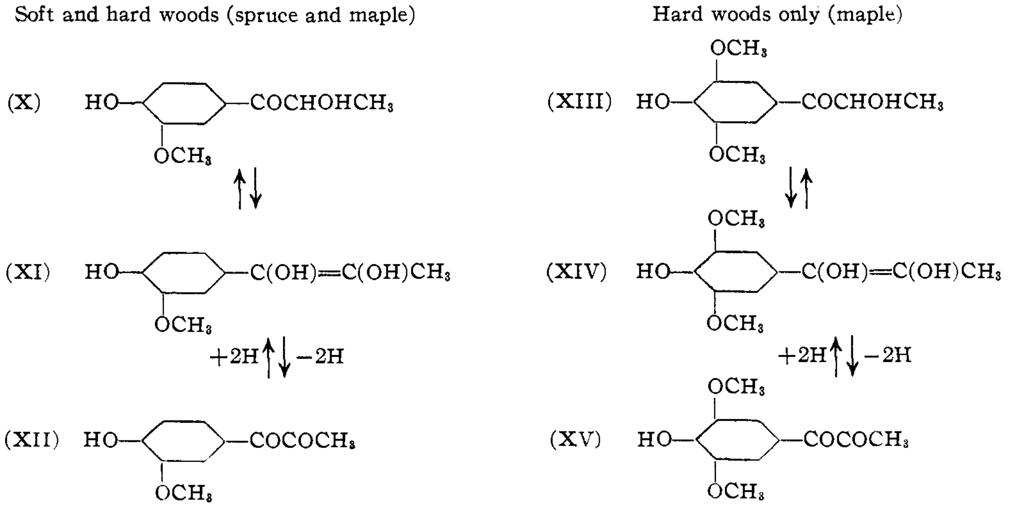


lin from vanilloyl methyl ketone, the scheme outlined in Chart I was found to provide a satisfactory process for the separation of these two components.

The amounts of vanillin and vanilloyl methyl ketone were found to vary considerably in different ethanolysis experiments. The results for three typical ethanolyses are given in Table I.

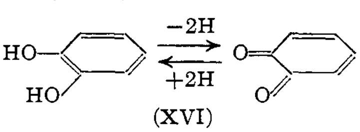
The two diketones (IV and VIII) can be regarded as members of two oxidation–reduction systems, the second members of which are the ene–diol forms of α-hydroxypropiovanillone and α-hydroxypropiosyringone, namely, 1-(4-hydroxy-3-methoxyphenyl)-1-propene-1,2-diol (α,β-dihydroxyisoeugenol) (XI) and 1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propene-1,2-diol (XIV), respectively. The dismutation forms, namely, α-hydroxypropiovanillone (X) and -syringone (XIII), have been isolated previously in the form of their α-ethyl ethers from the

OXIDATION–REDUCTION SYSTEMS

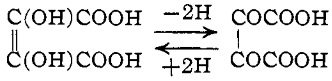


alkaline extract of the ethanolysis products of various woods.[7]

These oxidation–reduction systems are thus similar to those of pyrocatechol (XVI), dioxymaleic acid (XVII) and ascorbic acid (XVIII)

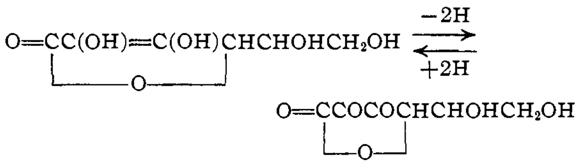


(XVI)



$$\begin{matrix}\mathrm{C(OH)COOH}\\ \|\\ \mathrm{C(OH)COOH}\end{matrix} \underset{+2\mathrm{H}}{\overset{-2\mathrm{H}}{\rightleftharpoons}} \begin{matrix}\mathrm{COCOOH}\\ |\\ \mathrm{COCOOH}\end{matrix}$$

(XVII)



$$\mathrm{O{=}CC(OH){=}C(OH)CHCHOHCH_2OH} \underset{+2\mathrm{H}}{\overset{-2\mathrm{H}}{\rightleftharpoons}} \mathrm{O{=}CCOCOCHCHOHCH_2OH}$$

(XVIII)

shown by Szent-Györgyi[8] to have their corresponding plant oxidases. Possibly these function as part of the respiratory mechanism in various forms of plant life.

It is suggested that the new oxidation–reduction system indicated above may also function in a similar manner, and that, on reaching maturity, lignification takes place within the cell, involving the deposition of these building units in a polymerized form. This lignin encrustant of the woody cells is then partly depolymerized during the ethanolysis process to regenerate the components of the oxidation–reduction system.

Experiments are in progress with the object of proving the existence of plant oxidases corresponding to the above two new systems.

Experimental

Ethanolysis of Spruce Wood.—A complete and detailed account of the ethanolysis procedure and fractionation of the water-soluble oils is given in a recent communication[9] and this was followed carefully in the present investigation. The pretreated spruce wood meal was refluxed with anhydrous ethanol containing 2% hydrogen chloride in an inert atmosphere (carbon dioxide) for forty-eight hours and the reaction mixture filtered. This and all subsequent operations were carried out under a carbon dioxide atmosphere. The ethanol filtrate was brought almost to neutrality by the addition of solid sodium bicarbonate, then concentrated under reduced pressure and precipitated into water. The lignin was removed by filtration, the aqueous filtrate concentrated under reduced pressure to about one-seventh of its volume and the solution and slight amount of deposited tarry material then continuously extracted with benzene.

The benzene solution was shaken with aqueous bisulfite solution (20%), the aqueous extract acidified with dilute sulfuric acid and back-extracted continuously with benzene. This benzene extract was used for the present investigations.

The benzene solution was concentrated under reduced pressure leaving 4.5 g. of a reddish-brown, very viscous residue. This on distillation gave two fractions (86% yield): (a) 3.6 g. (79.5%) of a clear yellow oil distilling at a bath temperature of 80–160° and a pressure of 0.005 mm.; and (b), 0.3 g. (6.5%) of a red resinous product distilling at bath temperature 250–350° and pressure 0.025 mm.

Investigation of the Low-Boiling Fraction

(a) **Action of Oxidizing Agents.**—To a solution of 0.05 g. of fraction (a) in 10 cc. of water was added 0.2 cc. of 3% hydrogen peroxide. After standing for twelve hours at room temperature, solid sodium bisulfite was added and the solution extracted with ether. Evaporation of the latter left 0.02 g. of a colorless crystalline solid which was sublimed twice at 125° (2 mm.); m. p. 205–206°. A mixed melting point with authentic vanillic acid showed no depression.

(b) **Formation and Separation of the Semicarbazone Mixture from Fraction (a).**—One gram of the distilled oil (a) was dissolved in 3 cc. of ethanol and 7 cc. of water and a mixture of 1 g. of semicarbazide hydrochloride and 1.5 g. of sodium acetate in 5 cc. of water then added. After standing for twenty-four hours at 0°, the mixture of crystalline semicarbazones which had formed was filtered; yield 0.72 g. This was boiled with 5–10 cc. of hot ethanol and the insoluble disemicarbazone of vanilloyl methyl ketone (A) removed by filtration of the hot solution. From the filtrate there separated out on cooling a mixture of vanillin semicarbazone and vanilloyl methyl ketone monosemicarbazone. A partial separation of the two was obtained by boiling this mixture with about 5 cc. of water and filtering the less soluble vanillin semicarbazone (B) from the hot solution. From the filtrate there crystallized out on cooling the monosemicarbazone of vanilloyl methyl ketone (C). (B) and (C) were purified by recrystallizing alternately from ethanol and from water to constant melting point. Purification of (A) was effected by boiling with ethanol, followed by hot water, and the product finally recrystallized from a solution in glacial acetic acid by addition of petroleum ether (30–50°). The melting points and analyses of these three compounds were as follows:

(A) **Disemicarbazone of Vanilloyl Methyl Ketone.**—Fine, colorless crystals; m. p., 241°. *Anal.* Calcd. for $C_{12}H_{16}O_4N_6$: C, 46.7; H, 5.2; N, 27.2; OCH_3, 10.1. Found: C, 46.4; H, 5.5; N, 26.9; OCH_3, 10.1.

(B) **Vanillin Semicarbazone.**—Colorless, needle-shaped crystals; m. p., 229°. *Anal.* Calcd. for $C_9H_{11}O_3N_3$: OCH_3, 14.9. Found: OCH_3, 14.8. A mixed m. p. with an authentic sample showed no depression.

(C) **Monosemicarbazone of Vanilloyl Methyl Ketone.**—M. p. 214–215°. *Anal.* Calcd. for $C_{11}H_{13}O_4N_3$:

---

[7] (a) Cramer, Hunter and Hibbert, THIS JOURNAL, **61**, 509 (1939); (b) Hunter, Cramer and Hibbert, *ibid.*, **61**, 516 (1939).

[8] Szent-Györgyi and co-workers, *Z. physiol. Chem.*, **254**, 147 (1938); *ibid.*, **255**, 57 (1938); *ibid.*, **258**, 147 (1939); *J. Biol. Chem.*, **90**, 385 (1931); for general review by Szent-Györgyi, see *Ber.*, **72A**, 53 (1939).

[9] Brickman, Pyle, McCarthy and Hibbert, THIS JOURNAL, **61**, 868 (1939).

C, 52.6; H, 5.18; N, 16.7; OCH_3, 12.3. Found: C, 52.9; H, 5.3; N, 16.8; OCH_3, 12.3.

(c) **Conversion of the Mono- into the Disemicarbazone of Vanilloyl Methyl Ketone.**—To 0.09 g. of the monosemicarbazone, dissolved in 15 cc. of hot ethanol, a mixture of semicarbazide hydrochloride (0.2 g.) and sodium acetate (0.3 g.) dissolved in 3 cc. of water was added. The solution was refluxed for five hours, the insoluble disemicarbazone removed by filtration of the hot solution, washed with hot ethanol and then with hot water; yield 0.03 g. It was recrystallized from glacial acetic acid–petroleum ether (30–50°); m. p. 241°. No change in melting point when mixed with the disemicarbazone, previously isolated, was observed.

(d) **Formation and Separation of the 2,4-Dinitrophenylhydrazones from the Low-boiling Fraction.**—The 2,4-dinitrophenylhydrazine solution was made[10] by triturating 0.4 g. of 2,4-dinitrophenylhydrazine with 21 cc. of concentrated hydrochloric acid, diluting to 100 cc. with distilled water and filtering after standing for twenty-four hours.

To 0.24 g. of the low-boiling product (Fraction (a)) dissolved in a mixture of 5 cc. of ethanol and 100 cc. of water was added 200 cc. of the dinitrophenylhydrazine solution. After standing at room temperature for two hours, the solution was filtered through a sintered glass funnel, the precipitated hydrazones washed well with cold water and then dried in a vacuum desiccator; yield 0.445 g.

The mixture of hydrazones was washed with 10 cc. of dry chloroform, in which it was almost completely soluble at room temperature. The small insoluble residue melted around 241–248°. It was recrystallized from hot dioxane; m. p. 261–262°. A mixed melting point with vanillin 2,4-dinitrophenylhydrazone showed no depression.

Evaporation of the chloroform solution left a sticky crystalline mass. This was boiled with 5 cc. of ethanol, the mixture cooled and the yellow crystalline hydrazone of vanilloyl methyl ketone filtered off and washed with another 5 cc. of ethanol; m. p. of residual product, 224–225°. Recrystallization from ethyl acetate followed by dioxane–petroleum ether (30–50°) raised the m. p. of the fine, orange yellowish-colored crystals to 226–227°. *Anal.* Calcd. for $C_{16}H_{14}O_7N_4$: C, 51.3; H, 3.75; N, 15.0; OCH_3, 8.3. Found: C, 51.4; H, 4.1; N, 15.1; OCH_3, 8.3.

Evaporation of the ethanol filtrate obtained above left a reddish-colored, uncrystallizable material which is at present being investigated. The quantitative results of three typical ethanolyses are summarized in Table I.

(e) **Methylation of the 2,4-Dinitrophenylhydrazone of Vanilloyl Methyl Ketone.**—To a solution of 0.21 g. of 2,4-dinitrophenylhydrazone of vanilloyl methyl ketone in 15 cc. of dioxane was added exactly one equivalent of diazomethane in dry ether. The solution was allowed to stand overnight, the solvent removed at room temperature under reduced pressure and the residue recrystallized from dioxane–petroleum ether (30–50°); yellowish orange-colored needle-shaped crystals; m. p. 194–195°. *Anal.* Calcd. for $C_{17}H_{16}O_7N_4$: C, 52.5; H, 4.12; N, 14.4; OCH_3, 15.95. Found: C, 52.8; H, 4.5; N, 14.6; OCH_3, 15.8.

Synthesis of Veratroylacetaldehyde.—Acetovanillone (20 g.) was methylated by the method used by Buck[11] for the preparation of veratric aldehyde; yield of acetoveratrone, 69%.

A mixture of acetoveratrone (12 g.) and ethyl formate (7.5 g.) was added to 1.5 g. of sodium wire suspended in 40 cc. of dry benzene.[4] After standing twelve hours, the sodium salt of veratroylacetaldehyde which formed was filtered off, washed well with benzene and ether and dried in the air; yield, 12 g.

Four grams of this sodium salt was dissolved in 10 cc. of water, acidified with dilute sulfuric acid and extracted with ether. Evaporation of the ether left 3 g. of a dark red oil which distilled at a bath temperature of 180–230° and 0.1 mm. pressure to give 0.9 g. of veratroylacetaldehyde as a clear yellowish oil. *Anal.* Calcd. for $C_{11}H_{12}O_4$: C, 63.5; H, 5.76; OCH_3, 29.8. Found: C, 63.6; H, 5.84; OCH_3, 30.1.

Its 2,4-dinitrophenylhydrazone was prepared in the manner already described and purified by recrystallization first from chloroform–petroleum ether (30–50°) and then from dioxane–petroleum ether (30–50°); fine, yellowish orange-colored crystals; m. p. 189–190°. *Anal.* Calcd. for $C_{17}H_{16}O_7N_4$: C, 52.5; H, 4.12; N, 14.4; OCH_3, 15.95. Found: C, 52.3; H, 4.4; N, 14.4; OCH_3, 16.05. A mixed melting point with the methylated 2,4-dinitrophenylhydrazone from the natural material showed a marked depression (19°).

Synthesis of Vanilloyl Methyl Ketone.—Copper sulfate crystals (14.4 g.) were dissolved in a mixture of pyridine (24 cc.) and water (12 cc.) by heating on the steam-bath. α-Hydroxypropiovanillone (5 g.) was added to this hot solution and the reaction mixture maintained at 100° for two hours. The flask was cooled, the contents poured into a slight excess of dilute hydrochloric acid and the solution extracted with ether. Evaporation of the ether left 4.3 g. of a reddish oil, b. p. 125° (0.2 mm.). The distillate, a clear yellow oil (3.5 g., 70%), slowly solidified on standing and was recrystallized from hot water and then from a mixture of ethanol and water (1:4); lemon-yellowish-colored needle-shaped crystals, m. p. 72–73°. *Anal.* Calcd. for $C_{10}H_{10}O_4$: C, 61.8; H, 5.16; OCH_3, 16.0. Found: C, 61.6; H, 5.27; OCH_3, 16.0.

The quinoxaline was prepared by heating 0.4 g. of the diketone with 0.22 g. of *o*-phenylenediamine dissolved in 10 cc. of ethanol for one hour on the steam-bath, concentrating the solution to 5 cc., and then adding water to precipitate the product; yield, 0.6 g. The quinoxaline was recrystallized from dilute ethanol; light yellow-colored hexagonal crystals; m. p. 162–163°. *Anal.* Calcd. for $C_{16}H_{14}O_2N_2$: C, 72.1; H, 5.26; N, 10.5; OCH_3, 11.65. Found: C, 71.8; H, 5.59; N, 10.8; OCH_3, 11.6.

The monosemicarbazone, disemicarbazone and 2,4-dinitrophenylhydrazone of this material were prepared in the manner already described. The melting points found were 215–216°, 241° and 226–227°, respectively. Mixed melting points with the corresponding derivatives of the natural material showed no depression. *Anal. Monosemicarbazone.* Calcd. for $C_{11}H_{13}O_4N_3$: C, 52.6; H, 5.18; N, 16.7; OCH_3, 12.3. Found: C, 52.9; H, 5.38; N, 16.8; OCH_3, 12.1. *Disemicarbazone.* Calcd. for $C_{12}H_{16}O_4N_6$: C, 46.7; H, 5.19; N, 27.3; OCH_3, 10.05. Found: C, 46.9; H, 5.6; N, 27.4; OCH_3, 10.4. *2,4-Dinitrophenylhydrazone.* Calcd. for $C_{16}H_{14}O_7N_4$: C, 51.3;

---

(10) Rubin and Bloom, *Am. J. Pharm.*, **108**, 387 (1936).

(11) Buck, "Organic Syntheses," Vol. XIII, 1933, p. 102.

H, 3.74; N, 14.95; OCH_3, 8.3. Found: C, 51.0; H, 4.2; N, 14.85; OCH_3, 8.4.

Acknowledgment.—Thanks are due to the Spruce Falls Power and Paper Company for the award of a Scholarship to one of us (L. B.).

Summary

1. The bisulfite-soluble fraction obtained from the ethanolysis of spruce wood has been shown to contain vanillin and a hitherto unknown dicarbonyl compound, vanilloyl methyl ketone.

2. Two new dicarbonyl compounds, veratroylacetaldehyde and vanilloyl methyl ketone have been synthesized and the latter shown to be identical with the naturally occurring material.

3. A quantitative separation of the components present in the bisulfite fraction has been effected by means of their 2,4-dinitrophenylhydrazones.

4. The amounts of vanillin and vanilloyl methyl ketone have been shown to vary in different ethanolysis experiments.

5. The possibility that vanilloyl methyl ketone may form one member of an oxidation–reduction system functioning in plant respiration is pointed out.

MONTREAL, CANADA RECEIVED MAY 6, 1940

[CONTRIBUTION FROM THE BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, UNITED STATES DEPARTMENT OF AGRICULTURE]

Constituents of Certain Species of Helenium. IV. Concerning the Compound Melting at 233–234° Obtained from Helenium tenuifolium[1]

BY E. P. CLARK

As incidental information in the second paper of this series[1a] a report was made upon a compound assigned the formula $C_{16}H_{22}O_5$ which melted at 233–234°. This substance was thought to be a constituent of the plant from which it was obtained, but further work has shown that it is an ether, $C_{19}H_{26}O_6$, formed as a result of a reaction between tenulin and ethylene glycol, the solvent used in its preparation.

The history of the material is interesting and instructive but it will suffice to say that while the analytical data obtained upon the substance and several of its derivatives indicated the C_{16} formula, the Rast molecular weight values were unreliable, and led to erroneous conclusions. The first indication of this appeared when, in the course of degradation experiments, acetyltenulinic acid, a C_{17} compound, was obtained.

The error is one that should be corrected and as the experiments which led to its discovery are pertinent to the tenulin problem, the work involved is now presented.

Because of the unreliability of the Rast method of determining molecular weights on this series of compounds a new value on the mother substance was sought that left no doubt as to its accuracy. This was done by introducing into the compound an accurately determinable alkoxyl group in the form of an ethoxy acetate. Upon analysis this was shown to have 15.04% of ethoxyl. When, however, the alkyl value (calculated as ethoxyl) given under identical conditions by the corresponding quantity of mother substance was subtracted from the above figure, the result was 10.3% of ethoxyl. This corresponds to a molecular weight of 437 for the acyl compound, and hence the molecular weight of the mother substance is 351. The calculated molecular weight for $C_{19}H_{26}O_6$ is 350.4. Attention is directed to the analytical data for this compound which has carbon and hydrogen values agreeing equally well for an ethoxy acetate of the formerly proposed C_{16} compound.

Attempts at degradation led to the formation of a monocarboxylic acid, $C_{19}H_{26}O_9$. This compound was obtained by two methods, alkaline hydrogen peroxide oxidation and permanganate oxidation. The acid obtained was purified readily and melted at 239°. It was found, however, that when an effort was made to recrystallize the acid from a boiling solution, by adding an excess of mineral acid to its sodium salt, a new material separated which melted at 319°. When an attempt was made to recrystallize this product, it changed to ordinary acetyltenulinic acid, m. p. 238–239°. Analysis of the acid melting at 319° gave a titration value corresponding to a molecular weight of 360, although carbon and hydrogen values were

---

[1] Not subject to copyright.

[1a] Clark, THIS JOURNAL, **61**, 1836 (1939).