[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

The Structural Identification of the Olefinic Components of Japanese Lac Urushiol[1]

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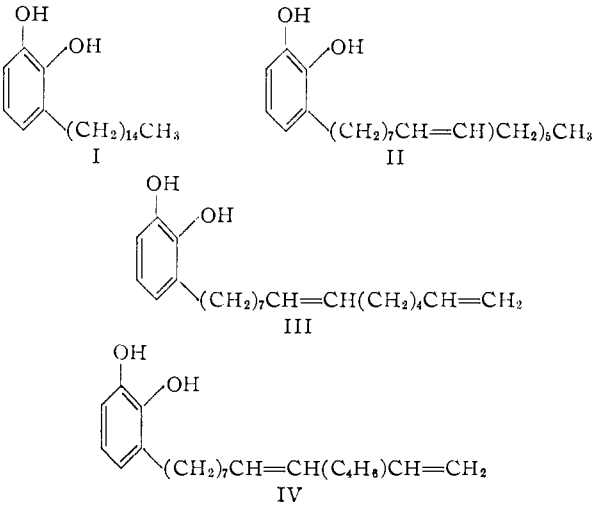
The heteroölefinic composition of urushiol, the vesicant principle of Japanese lac, has been confirmed. By means of chromatography on alumina the dimethyl ether has been separated into four components whose structures have been established. All of the components of urushiol have the carbon skeleton of 3-pentadecylcatechol. A monoölefin, diolefin and triolefin account for about 95% of the urushiol. The fourth component has the saturated side chain. The structures of the di- and triolefinic components have been found to be different from those proposed earlier by Majima. The major difference between the alkenyl catechols of Japanese lac and poison ivy is found in the triolefinic components. The lac triolefin, which constitutes about 50% of urushiol, possesses a conjugated diene system. The corresponding triolefin of poison ivy has no conjugation.

It has recently been found in these laboratories that the alkenyl phenols present in poison ivy extract and in cashew nut shell liquid are heteroolefinic in composition. By means of chromatographic adsorption on alumina, employing the methyl ethers, the various olefinic components differing only in degree and in position of unsaturation have been separated and structurally identified.[3,4] These results have focused attention on earlier investigations concerning the heteroölefinic composition of urushiol, the alkenyl phenol found in the sap of the Japanese lac tree (*Rhus verniciflua*).

For many years Japanese lac has been a material of commercial interest because of its use in the preparation of decorative and protective coatings for wooden articles manufactured in Japan. Interest in this material also has been stimulated by its vesicant properties. As the result of structural studies, extending over a period of about 15 years, Majima and co-workers established that the carbon skeleton of the active principle (urushiol) in Japanese lac is that of catechol with a normal 15 carbon side-chain in the 3-position.[5]

Because the dimethyl ether of the toxic oil had an unsaturation equivalent to about two olefinic bonds, and yielded a variety of products on ozonolysis and other oxidative degradation procedures, Majima concluded that urushiol was probably a mixture of four compounds which could not be separated by fractional distillation. He showed that the side-chain of one of the compounds was saturated, and postulated that the other three components of urushiol differed only in the number of double bonds contained in the alkenyl side-chain. He proposed the following structures for the four components of urushiol.[5]

Since Majima was not able to separate the olefinic components of dimethylurushiol in pure form prior to the oxidative degradations, the structures he proposed for the di- and triolefinic components in order to account for the variety of degradation products are not unequivocal. His attempts to isolate pure di- and tri-ozonides following controlled ozonization of urushiol dimethyl ether were not successful judging from the analytical data on the



ozonides and their hydrolytic products. For example, each of these ozonides gave heptaldehyde, heptoic acid and ω-(dimethoxyphenyl)-caprylic aldehyde on hydrolysis, products which were presumably derived from the ozonide of the monoolefin.

In 1934, Hill and co-workers[6] concluded that "the toxic principle of poison ivy is urushiol," a view that has been generally accepted.[7,8] However, Hill's experiments only proved that the poison ivy principle and urushiol had the same carbon skeleton, that of 3-pentadecylcatechol. Recently[4b] the structures of the olefinic components of the poison ivy principle have been elucidated and the diolefinic component has been shown to have a structure different from that proposed by Majima for the diolefinic component of the lac urushiol. In view of this circumstance, and the fact that Majima was not able to propose a complete structure for the triolefinic component, it has seemed advisable to reinvestigate the olefinic components of lac urushiol.

Dimethylurushiol, prepared from Japanese lac according to the procedure of Majima,[9] was purified by careful fractional distillation and then subjected to chromatography on activated alumina for the purpose of separating the various components. The chromatographic procedures and techniques were similar to those previously described,[4b] but

(1) This investigation was supported by a grant from the Lederle Laboratories Division of the American Cyanamid Company. It is a pleasure to acknowledge their interest and assistance.

(2) Postdoctoral Research Associate from the University of Bombay, India.

(3) W. Symes and C. R. Dawson, *Nature*, **171**, 841 (1953).

(4) (a) W. Symes and C. R. Dawson, THIS JOURNAL, **75**, 4952 (1953); (b) **76**, 2959 (1954).

(5) R. Majima, *Ber.*, **55B**, 172 (1922).

(6) G. A. Hill, V. Mattacotti and W. D. Graham, THIS JOURNAL, **56**, 2736 (1934).

(7) F. A. Stevens, *J. Am. Med. Assoc.*, **127**, 912 (1945).

(8) W. M. Harlow, "Poisonivy and Poisonsumac." New York State College of Forestry at Syracuse. N. Y., Vol. xxii, No. 4 (1949).

(9) R. Majima, *Ber.*, **42**, 1418 (1909).

the chromatographic separation of the lac dimethylurushiol turned out to be more complicated than in the case of the poison ivy dimethyl-"urushiol." The main complication was the formation of a yellow band in the upper regions of the alumina column, even though the lac dimethylurushiol and the column were kept at all times under an atmosphere of nitrogen previously purified by passing it through a solution of alkaline pyrogallol.

As found in the previous investigation,[4b] the saturated component, 3-pentadecylveratrole, was the least adsorbed to the alumina and therefore was the first to appear in the eluant during the development of the column. At this point the column was extruded, arbitrarily sectioned, and the adsorbed material on each section recovered by extraction with diethyl ether. The refractive indices of the recovered oils gave an indication of the degree of separation of the components. Samples of the pure mono- and diolefinic components were obtained by repeated chromatographic separations employing fractions of the appropriate region of refractive indices. The purity of the mono- and the diolefinic components was judged by means of catalytic hydrogenation. Both of these components absorbed the theoretical amount of hydrogen and yielded pure 3-pentadecylveratrole of sharp melting point (34–35°).

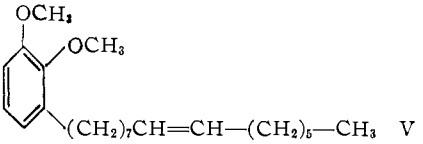
The triolefinic component, however, could not be purified in this way. On repeated chromatographic separations, it became more and more yellow and appeared to be converted in part into a polymeric product, possibly as the result of autoxidation. The presence of agents such as sodium hydrosulfite or pyrogallol at the top of the column during the chromatographic separations did not prove helpful. Finally it was found that the triolefinic component could be separated from the yellow contaminants by chromatography on a column of Florisil.[10] Although this adsorbent is much less active than alumina and is not suitable for the preliminary separation of dimethylurushiol into its components, it proved to be very satisfactory for removing the colored impurities from the triolefinic component. The triolefin thus purified gave on catalytic hydrogenation a double bond value of 3.0 and yielded quantitatively 3-pentadecylveratrole of sharp melting point.

Whereas the refractive indices of the saturated, monoölefinic and diolefinic components of the lac dimethylurushiol were found to be the same as those of the corresponding components of poison ivy dimethyl-"urushiol,"[4b] the refractive index of the triolefinic component of Japanese lac dimethylurushiol (n^{25}D 1.5225–1.5230) was found to be significantly higher than that of the triolefin of poison ivy dimethyl-"urushiol" (n^{25}D 1.5145–1.5175). This fact, and the sensitivity to alumina, mentioned above, suggested a fundamental difference in the structure of the side-chains of the two olefins.

More information concerning the structural differences between the triolefinic components of the lac and the poison ivy dimethylurushiol was obtained from their ultraviolet spectra in 95% ethanol (1×10^{-4} *M*). The spectrum of the lac triolefin differed strikingly from that of the poison ivy triolefin. A strong band having a maximum at 2270 Å. (ϵ 2.35×10^4) was characteristic of the lac triolefin whereas the poison ivy triolefin showed no band at this wave length (ϵ 0.65×10^4). The 2270 Å. band was also observed in the spectrum of the original lac dimethylurushiol but was not present in the spectra of the purified saturated, mono- and diolefinic components. The latter spectra were very similar to those of the components of poison ivy dimethylurushiol and methylcardanol.[4] The band at 2270 Å. can be attributed to conjugation in the side chain of the lac triolefin. The position and intensity of the band are characteristic of a conjugated diene,[11] indicating that the third double bond is probably unconjugated. It is well known that conjugated trienes exhibit an intense absorption band at 2700 Å.[12]

Information concerning the geometrical configuration of the olefinic bonds of each of the components was obtained by examining their infrared spectra. The absence of a band at 10.3 μ is characteristic of the *cis*-olefin configuration.[13] The purified monoölefinic component showed no band at 10.3 μ and therefore it has been assigned the *cis* configuration. The di- and the triolefinic components, however, showed a little absorption at 10.3 μ. It would appear, therefore, that these components were mainly in the *cis* configuration but contained small amounts of *trans* isomers. This point is related to the fact that the di- and the triolefinic components showed some variation in the refractive indices of the final fractions obtained during their chromatographic purification.

To establish the positions of the double bonds, each of the pure olefinic components obtained by chromatography of lac dimethylurushiol was ozonized and the resulting ozonides were catalytically hydrogenated to give the corresponding aldehydes. The monoölefin gave heptaldehyde and ω-(2,3-dimethoxyphenyl)-caprylic aldehyde, which was characterized by conversion into the amide and anilide of the corresponding acid. Hydroxylation of the monoölefin with osmium tetroxide gave a crystalline glycol which proved to be identical (mixed m.p.) with the glycol similarly obtained from the monoölefinic component of the poison ivy dimethylurushiol. This established that the two monoölefins are structurally identical, both possessing the structure of *cis*-3-(pentadecenyl-8)-veratrole (V).



V

The diolefinic component on ozonolysis yielded butyraldehyde, malondialdehyde and ω-(2,3-dimethoxyphenyl)-caprylaldehyde. The latter was identified as previously described and the malondi-

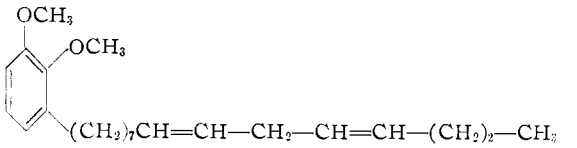
(10) A synthetic magnesia–silica gel supplied by the Floridin Company of Warren, Penna.

(11) R. B. Woodward, THIS JOURNAL, **64**, 72 (1942).

(12) M. G. Mellon, "Analytical Absorption Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 402.

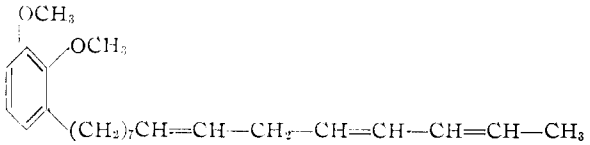
(13) B. Loev and C. R. Dawson, to be published.

aldehyde was characterized as the dimedon derivative. The structure of the diolefin is, therefore, established as 3-(pentadecadienyl-8′,11′)-veratrole (VI)

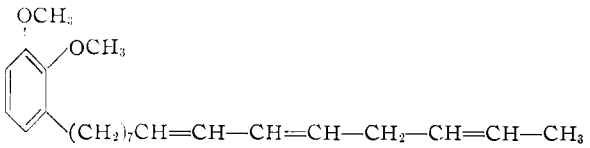


VI

From the ozonolysis of the triolefin, acetaldehyde, malondialdehyde and the caprylic aldehyde were isolated. Two structures can be proposed for a triolefin that will account for these degradation products.



VII



VIII

Because of the conjugation of two of the double bonds in the side-chain, both of these structures would be expected to give an ultraviolet absorption spectrum characterized by a high intensity band at 2270 Å. such as observed with the pure triolefinic component.

In order to establish the position of the conjugation, the maleic anhydride adduct of the triolefin was prepared. The adduct, after exhaustive extraction with ligroin to remove any of the unreacted triolefin, was ozonized. Catalytic reduction of the ozonide produced ω-(2,3-dimethoxyphenyl)-capryl aldehyde, the product to be expected from the maleic anhydride adduct of VII. All attempts to recover acetaldehyde, the product to be expected from the maleic anhydride adduct of VIII, were unsuccessful. It may be concluded, therefore, that the arrangement of the double bonds in the side-chain of the triolefinic component is that represented by VII.

It is to be noted that the structures of the diolefinic component VI and the triolefinic component VII of Japanese lac urushiol as established in this investigation differ from those proposed earlier by Majima[5] (see III and IV). In this connection it is pertinent to re-emphasize the fact that Majima did not have the pure olefinic components available for structural investigation.

The terminal double bonded structure III proposed by Majima[5] for the diolefin was based on the isolation of a small amount of adipic acid from the potassium permanganate oxidation of a large sample of the mixture of olefins (dimethylurushiol) and also on the isolation of formaldehyde and formic acid as ozonolysis products. In the present investigation, repeated attempts to find these products among those obtained from the ozonolysis of the pure diolefin have been unsuccessful. It would seem, therefore, that they occurred in Majima's experiments either as the result of impurities present in his dimethylurushiol or as the result of side reactions. It is well known that the hydrolysis procedure Majima employed for the ozonide decompositions leads to both aldehydes and carboxylic acids, because of secondary reactions involving hydrogen peroxide. It would seem possible, therefore, that under such conditions the formaldehyde and formic acid found by Majima may have resulted from further oxidative degradations involving glyoxal derived from the triolefin VII. As a matter of fact, Majima was not certain of the existence of a diolefinic component and suggested that the properties of dimethylurushiol and its degradation products might be explained in terms of a mixture of saturated, monoölefinic and triolefinic components.

Because of the occurrence of formaldehyde and formic acid on ozonolysis, Majima also proposed a terminal double bonded structure IV for the triolefinic component. The 8′-position of a double bond in all of the olefinic components agreed with the isolation of ω-(2,3-dimethoxyphenyl)-caprylaldehyde (acid) as the only ozonolysis products containing an aromatic ring. In the case of the triolefinic component, Majima was not able to locate the position of the intermediate double bond.

As pointed out previously, no evidence has been obtained in this investigation for a terminal double bond in either the di- or the triolefinic components. The infrared spectra of these pure components do not show the absorption bands at 6.05 and 11.0 μ which are characteristic of a vinyl group. These bands are also not present in the infrared spectrum of dimethylurushiol. The structures VI and VII are in agreement with this aspect of the infrared spectra.

It is of considerable interest to note that the structure of the triolefinic component of lac urushiol is different from that of the corresponding component of the poison ivy principle. The triolefin of poison ivy "urushiol" is unconjugated and does possess a terminal double bond.[4b] Repeated attempts to find such a triolefin in the lac dimethylurushiol have been unsuccessful.

Experimental[14,15]

Purification of Crude Japanese Lac.[16]—A 200-g. sample of crude Japanese lac was shaken well in 1200 ml. of ethanol, and the dark brown solution was set aside to allow the suspended insoluble material to settle. The alcoholic solution was filtered and the gray residue (13 g.) was washed with 100 ml. of ethanol. The dark brown oily residue (147 g.), obtained after removing the solvent under reduced pressure, was dissolved in 400 ml. of petroleum ether (b.p. 30–60°). The resulting solution was washed with water and dried over calcium chloride. It was then diluted with 8 liters of petroleum ether and allowed to stand overnight. The separated waxy material was filtered off and the solvent was removed by distillation. The residual oil weighed 117 g. (58.5%), and gave an intense olive-green ferric chloride test.

(14) All melting points are corrected.

(15) Microanalyses were performed by Tiedcke Laboratory of Microchemistry, Teaneck, N. J., and Schwarzkopf Microanalytical Laboratory, Middle Village, L. I., N. Y.

(16) Supplied by the Lederle Laboratories Division of the American Cyanamid Company.

Dimethylurushiol.—A 90-g. sample of the above solvent purified urushiol was methylated with methyl iodide according to the procedure of Majima.[9] The yield of the crude methylated product was 74 g. (76%). It gave a negative ferric chloride test. The crude dimethyl ether was distilled and after a 7-g. forerun a fraction boiling at 184–187° (0.2–0.3 mm.) was taken; 42.0 g. (43%), $n^{25}D$ 1.5135. The residue in the distillation flask polymerized into a dark-brown product. A 26.0-g. sample of the above distillate was carefully refractionated at 0.04 mm. into three fractions of pale yellow color; A, b.p. 130-168°, 5.0 g.; B, b.p. 168–172°, $n^{25}D$ 1.5135, 10 g.; C, b.p. 172–178°, $n^{25}D$ 1.5185, 10 g. On catalytic hydrogenation fraction B absorbed hydrogen equivalent to 2.4 double bonds and gave a quantitative yield of pure 3-pentadecylveratrole, m.p. 32–34° without recrystallization. Fraction C gave a double bond value of 2.72 but the resulting hydrogenated product was a waxy material indicating the presence of impurities. Pure 3-pentadecylveratrole was obtained from this material by one crystallization from methanol.

Chromatographic Separation of Dimethylurushiol.—The 10 g. of dimethylurushiol (fraction B above) was dissolved in 50 ml. of ligroin (b.p. 60–80°) and the solution was passed through a column of 700 g. of activated alumina (grade I)[4a,17] of dimensions 6 × 36 cm. The chromatogram was developed with ligroin under a steady pressure of 5 lb. of nitrogen, previously deoxygenated by passing it through an alkaline pyrogallol solution. The rate of flow of the effluent was 15 liters per hour. After one hour, the development of the column was stopped. The alumina was extruded by nitrogen pressure and sectioned arbitrarily into 23 portions. Each section was then extracted with anhydrous diethyl ether[18] to recover the adsorbed material. After removing most of the ether by distillation, the residual traces of the solvent were removed under nitrogen by careful heating (50–60°). The residues were combined according to the similarity of their refractive indices to give four fractions: (A) 0.5 g., $n^{25}D$ 1.4905–1.4925 (5–8%); (B) 1.83 g., $n^{25}D$ 1.4940–1.4980 (20–25%); (C) 0.96 g., $n^{25}D$ 1.5000–1.5040 (10–15%); (D) 4.36 g., $n^{25}D$ 1.5225–1.5230 (45–50%). The olefinic components thus obtained from two or three similar chromatographic separations of dimethylurushiol were combined and rechromatographed repeatedly until satisfactorily pure samples of each of the olefins were obtained. The refractive indices of the purified mono-, di- and triolefins thus obtained were $n^{25}D$ 1.4940, 1.5030–1.5050 and 1.5225–1.5230, respectively. Each of the olefins absorbed the theoretical amount of hydrogen on catalytic reduction and yielded pure 3-pentadecylveratrole. The saturated component (A) after recrystallization from methanol melted at 35–36°, which was not depressed when it was mixed with 3-pentadecylveratrole.

Ozonolysis of the Monoölefin.—A solution of 550 mg. (0.0016 mole) of the chromatographically pure monoolefin ($n^{25}D$ 1.4940) in 25 ml. of ethyl acetate was ozonized at −80° and the ozonide was hydrogenated and processed as described earlier.[4a] The heptaldehyde obtained in the steam-volatile fraction was characterized as the 2,4-dinitrophenylhydrazone, obtained in 39% yield, m.p. 104–104.5°, not depressed when mixed with an authentic sample.

The aromatic fragment remaining in the oily residue from the steam distillation was transferred into acetone and oxidized with potassium permanganate (0.45 g.) at 40–50° as previously described.[4a] The resulting acid was converted into its acid chloride by treatment with purified thionyl chloride (3 ml.) in 30 ml. of ligroin for 30 minutes at 60–70°. After removing the solvent and excess thionyl chloride by distillation under reduced pressure the acid chloride was converted into the anilide by treatment with 0.8 g. of aniline dissolved in benzene. The crude crystalline product (0.35 g.) was treated with hot ligroin to extract the aniline. On cooling, a yield of 0.21 g. (37.5%) of cream colored crystalline anilide was obtained, m.p. 83–85°. On two further recrystallizations from ligroin the melting point rose to 91–92°.

Anal. Calcd. for $C_{22}H_{29}NO_3$: C, 74.33; H, 8.22. Found: C, 74.00, 74.20; H, 8.12, 8.31.

(17) H. Brockmann and H. Sckodder, *Ber.*, **74B**, 73 (1941).

(18) Colorless extracts were obtained using anhydrous ether, whereas ordinary U. S. P. ether also extracted the undesired yellowish impurities formed on the column presumably by autoxidation.

Ozonolysis of the Diolefin.—A solution of 700 mg. (0.00204 mole) of chromatographically pure diolefin ($n^{25}D$ 1.5030) in 25 ml. of ethyl acetate was ozonized at −80°. On catalytic reduction the ozonide absorbed about 60% of the theoretical amount of hydrogen. The ethyl acetate solution of the reduced ozonide was then extracted with 30 ml. of water. To the aqueous extract were added 2 g. of methone (5,5-dimethyldihydroresorcinol), 30 ml. of absolute alcohol and a drop of piperidine. The mixture was refluxed gently for 10 minutes. On cooling the reaction mixture, a colorless precipitate separated, 100 mg. (22%),[19] m.p. 220–225°. It was purified by three crystallizations from ethanol. The colorless crystalline compound melted at 243–244° with decomposition. A mixed m.p. with an authentic sample of the tetramethone of malondialdehyde showed no depression. The reported m.p. for this compound is 234–237°.[20] For the identification purposes the malondialdehyde was prepared by the ozonolysis of linoleic acid, and the resulting water-soluble aldehyde was converted into its tetramethone, m.p. 243.4–244.5°, in 30% over-all yield.

Anal. Calcd. for $C_{35}H_{48}O_8$: C, 70.32; H, 8.10. Found: C, 70.60, 70.41; H, 7.99, 8.00.

The ethyl acetate layer, separated from the aqueous extract, was distilled directly into 25 ml. of ethanol containing 1.4 g. of methone reagent and one drop of piperidine. The resulting mixture was refluxed for five minutes and cooled. No precipitation occurred and the solvent was, therefore, removed by evaporation and the orange-yellow residue crystallized from methanol, 180 mg. (48%),[19] m.p. 90–95°. After repeated crystallization the methone derivative melted at 125–128° and the cyclized form of the methone derivative (prepared by refluxing an ethanol solution with a drop of concd. hydrochloric acid) melted at the same temperature. The methone derivative of an authentic sample of butyraldehyde melted at 134–135°, and its cyclized form also melted at 135–136° as previously described.[21] A mixed melting point of the authentic butyraldehyde methone derivative and that obtained above (m.p. 125–128°) melted at 125–128°. In view of this difficulty in purifying the methone derivative of the water-insoluble volatile aldehyde, the 2,4-dinitrophenylhydrazone derivative was prepared, m.p. 118–119°; when mixed with an authentic sample of the 2,4-dinitrophenylhydrazone of butyraldehyde no depression in the m.p. was observed.

The aromatic fragment, remaining after the ethyl acetate distillation, was oxidized with potassium permanganate (0.5 g.) and the resulting acid was converted into the anilide in 44% over-all yield.[19] The purified anilide melted at 90–91.5°. A mixed m.p. with ω-(2,3-dimethoxybenzene)-capryl anilide was 91–92°. In an alternate experiment, the acid was converted into amide, m.p. 95–96°, which was undepressed when the amide was mixed with an authentic sample[4b] of ω-(2,3-dimethoxyphenyl)-capryl amide.

Ozonolysis of the Triolefin.—A solution of 1.0 g. (0.0029 mole) of the chromatographically pure triolefin was dissolved in 25 ml. of ethyl acetate and was ozonized as before. On catalytic reduction the ozonide absorbed 60% of the theoretical amount of hydrogen. The solution was distilled on a steam-bath and the distillate was collected directly under an ethyl acetate trap. To the distillate were added 2.0 g. of methone reagent and one drop of piperidine. The mixture was shaken well and allowed to stand overnight. It was then refluxed gently to complete the reaction. On cooling the reaction solution no precipitate separated. The solvent, therefore, was removed by evaporation. The yellowish crystalline residue was extracted with 15 ml. of methanol. The residue, 200 mg. (25%),[19] melted at 225–229°. After three recrystallizations from methanol the product melted at 243–244°. A mixed m.p. with an authentic sample of malondialdehyde tetramethone was 242–243.5° dec.

Anal. Calcd. for $C_{35}H_{48}O_8$: C, 70.32; H, 8.10. Found: C, 70.61; H, 8.25.

From the ethanol mother liquor 150 mg. (36%) of the methone of acetaldehyde was recovered. Because its m.p. was about ten degrees low even after repeated recrystalli-

(19) The yields of the derivatives are based on the % of ozonide hydrogenolyzed.

(20) D. Vorländer, *Z. anal. Chem.*, **77**, 241 (1929).

(21) E. C. Horning and M. G. Horning, *J. Org. Chem.*, **11**, 95 (1946).

zations, the derivative was cyclized by refluxing for 5 minutes 100 mg. in 3 ml. of 80% ethanol containing a drop of concd. hydrochloric acid. The second derivative of the methone, thus prepared, melted at 174.5–175.5° without crystallization. A mixed m.p. with an authentic sample of the second methone derivative of acetaldehyde showed no depression. The acetaldehyde was also recovered and identified in the form of its 2,4-dinitrophenylhydrazone.

The original residue which was left after the ethyl acetate distillation was dissolved in ethyl acetate (15 ml.) and the solution was extracted with 25 ml. of water. From the aqueous layer 50 mg. of the tetramethone of malondialdehyde was obtained, m.p. 243–244°. The total yield of the tetramethone of malondialdehyde was 310 mg. (30%).[19]

The aromatic fragment obtained from the ethyl acetate layer was oxidized with potassium permanganate (0.5 g.) and the resulting oily acid was converted into its amide in 42% over-all yield.[19] The m.p. of the pure sample was 96°, and a mixed m.p. with an authentic sample[4b] of ω-(2,3-dimethoxyphenyl)-capryl amide was 96–97°. In an alternate experiment, the resulting acid was characterized as its anilide, m.p. 91–92°. A mixed m.p. with an authentic sample of ω-(2,3-dimethoxyphenyl)-capryl anilide showed no depression.

Anal. Calcd. for $C_{22}H_{29}NO_3$: C, 74.33; H, 8.22; N, 3.94. Found: C, 74.18; H, 8.25; N, 4.09.

Maleic Anhydride Adduct of the Triolefin.—A solution of 1.5 g. (0.0044 mole) of the triolefin, 0.48 g. (0.0049 mole) of maleic anhydride and a trace of hydroquinone in 25 ml. of benzene was refluxed for 24 hours under a nitrogen atmosphere. The solvent was distilled off and the unreacted maleic anhydride was removed by sublimation under reduced pressure. The yellowish oily residue was dissolved in benzene (10 ml.) and ligroin was then added to precipitate the adduct. The solvent was removed by decantation and the gummy precipitate when stirred into petroleum ether soon solidified. The yield was 0.85 g. (43%). It was crystallized from benzene–ligroin. The product, however, did not melt sharply. The m.p. of the compound was 95–100°. It was therefore extracted (soxhlet) with ligroin for 5 hours to remove any soluble impurities, especially traces of unreacted triolefins.

Ozonolysis of the Adduct.—A solution of 0.75 g. (0.0017 mole) of the maleic anhydride adduct was cooled to −80°, and ozonized as previously described. The resulting ozonide was reduced catalytically over palladium-on-calcium carbonate. It absorbed 54% of the theoretical amount of hydrogen. The ethyl acetate solution of the reduced ozonide was extracted with 40 cc. of water. To the aqueous layer was added a freshly prepared solution of 0.4 g. of 2,4-dinitrophenylhydrazine. There was no evidence of any hydrazone formation and all attempts to isolate the hydrazone of acetaldehyde were unsuccessful. The ethyl acetate layer was concentrated on a steam-bath to a yellowish oily residue, which was dissolved in acetone (25 ml.) and oxidized with potassium permanganate (0.5 g.). The resulting acidic material, subsequently identified as ω-(2,3-dimethoxyphenyl)-caprylic acid, was isolated as previously described. It was identified by converting into the amide which after two recrystallizations from ligroin melted sharply at 95–96° (20 mg.). A mixed m.p. with an authentic sample of ω-(2,3-dimethoxyphenyl)-capryl amide showed no depression.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE MARITIME REGIONAL LABORATORY, NATIONAL RESEARCH COUNCIL OF CANADA]

Degradative Studies on Fucoidin[1]

BY A. N. O'NEILL

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Fucoidin, prepared from *Fucus vesiculosus*, was subjected to mild acetolysis and the material recovered from the acetolysate was converted to a mixture of acetylated alditols. Chromatographic resolution of this mixture on Magnesol has led to the isolation in crystalline form of L-fucitol pentaacetate and the acetylated derivative of a reduced disaccharide. This disaccharide derivative has been converted to the crystalline free sugar alcohol. This has been defined by analysis and periodate oxidation as 2-α-L-fucopyranosyl-L-fucitol, thereby providing further evidence for the presence of 1,2-glycosidic linkages in fucoidin.

Fucoidin is the chief polysaccharide sulfate ester of the *Phaeophyceae* where it occurs together with alginic acid, laminarin and mannitol. It was first described by Kylin[2] who isolated the polysaccharide from various species of *Laminaria* and *Fucus* by extraction with dilute acetic acid and proved the presence of L-fucose in a hydrolysate by isolating the phenylosazone. Bird and Haas[3] obtained crude fucoidin by aqueous extraction of the fronds of *Laminaria digitata* followed by precipitation with ethanol. Purification yielded a product containing 30.9% ash and 30.3% sulfate. Since the total sulfate found on hydrolysis was approximately double that found in the ash, fucoidin was considered to be the salt of a carbohydrate half ester of sulfuric acid.

More recent structural studies have been carried out on this polysaccharide.[4–6] It was noted by Percival and Ross[5] that, even after drying at 40° and a pressure of 0.1 mm. for a considerable length of time, fucoidin still retained 9.4% water and 6% alcohol. After correction for adsorbed solvents their analysis was as follows: sulfate, 38.3%; metals, 8.2%; uronic acid, 3.3%; fucose, 57%; galactose, 4.1%; xylose, 1.5%. The calcium salt of a fucan monosulfate would give sulfate, 39.2%; calcium, 8.2%; fucose, 66.9%. Fucoidin was believed to be a fucan monosulfate and that constituents other than fucose resulted from impurities in their preparation.

Methylation studies[6] combined with a study of the stability of the sulfate residue to alkaline hydrolysis indicated that fucoidin could be represented by a chain of L-fucopyranose units joined by α-glycosidic linkages through carbon atoms one and two of adjacent units, each fucopyranose unit carrying a sulfate on carbon four. From the data on methylation it is obvious that there is a high proportion of other linkages present.

In the present investigation an attempt has been made to define this polysaccharide more accurately through a study of the products resulting on partial hydrolysis.

Fucoidin was prepared from fresh *Fucus vesiculosus* by the method of Black, Dewar and Wood-

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