Even in very low concentrations, both acetate and phosphate increase the activity of malt amylase slightly.

Acetate, well known to be much the more efficient buffer in the range of hydrogen-ion activities suitable for work with this enzyme, is here found to be as effective in activating the enzyme as is phosphate, and to be experimentally applicable over a wider range of concentration.

Acetate is, therefore, preferable to phosphate as a buffer salt for use with malt amylase.

In an acetate concentration of 0.01 *M* the optimal activity was found at *P*H 4.5 to 4.8, and in a concentration of 0.1 *M*, at *P*H 5.0 to 5.4.

NEW YORK, N. Y.

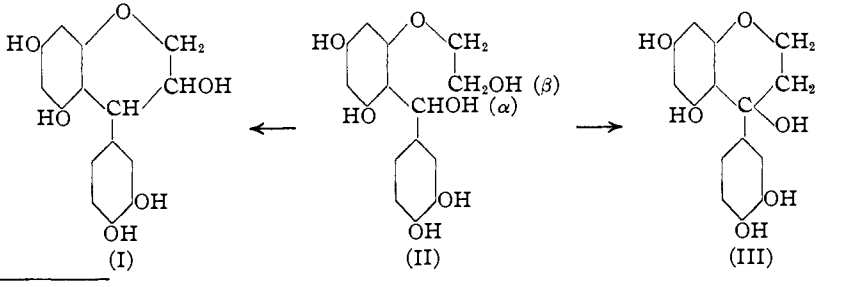
[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF BRISTOL]

THE CONSTITUTION OF CATECHIN. X

BY M. NIERENSTEIN

RECEIVED DECEMBER 26, 1929 PUBLISHED APRIL 7, 1930

Only the heart-wood of the cutch-producing acacias,[1] *Acacia Catechu*, Willd., *A. Catechuoides*, Benth. and *A. Sundra*, D. C. is used in the manufacture of cutch, since it is generally believed that the other parts of the plant contain no catechin.[2] An examination of the sap-wood, bark and young twigs of these three acacias, carried out in this Laboratory, supports this generally accepted view. Furthermore, it was found that the catechin content of the heart-wood increases as the medulla is approached, which would seem to indicate that catechin is a final product of metabolism in these acacias,[3] and must therefore be derived from some other product formed in the living plant. This hypothesis is confirmed by the presence of *l*-leucomaclurin-glycol ether (II) in the young twigs of *Acacia Catechu*, Willd. The production of acacatechin (I) and iso-acacatechin (III) from *l*-leucomaclurin-glycol ether (II) would then follow as a matter of course.



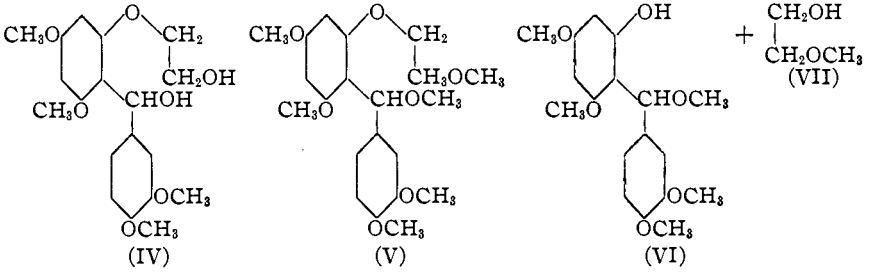
(I) (II) (III)

[1] Prain, *J. Asiatic Soc. Bengal*, **66**, 508 (1897).

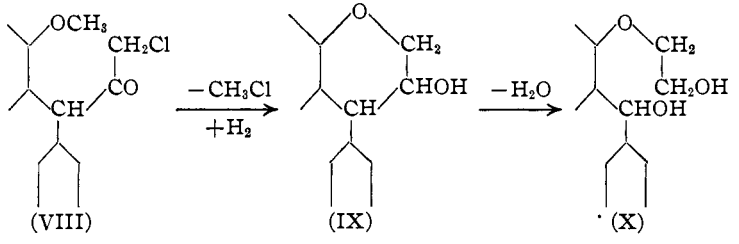
[2] Wiesner, "Die Rohstoffe des Pflanzenreiches," 3d ed., Leipzig und Berlin, **1914**, Vol. I, p. 605.

[3] These considerations obviously do not apply to gambier-catechin present in the leaves of *Umcaria Gambier* and allied species.

Substance II yields on methylation with diazomethane a tetramethyl ether to which Formula IV can be assigned with some degree of certainty, since the two aliphatic hydroxyl groups marked α and β, respectively, would not react with diazomethane.[4] . On methylation of Substances II and IV with methyl iodide and silver oxide, the hexamethyl ether (V) is produced, which yields on hydrolysis pentamethyl-leucomaclurin (VI) and monomethyl-glycol (VII).



On acetylation by heating with acetic anhydride *l*-leucomaclurin-glycol ether (II) yields penta-acetyl-*l*-acacatechin (I), this being further evidence for the constitution of acacatechin, which is produced from *l*-acacatechin on racemization. The acacatechin nucleus, (IX), has thus been built up along two different lines: (1) by ring closure of (VIII) followed by reduction of the carbonyl group as recorded in 1921 and 1926;[5] (2) by ring closure of (X) as described in the present communication. These results obviously substantiate no other formula for catechin but that suggested by the writer in 1920.[6]



Reference must be made to the fact that although during the conversion of *l*-leucomaclurin-glycol ether into penta-acetyl-*l*-acacatechin a new asymmetric carbon atom is produced, no other product but penta-acetyl-*l*-acacatechin could be detected. This can only be accounted for by the

[4] With reference to hydroxyl group α see: Nierenstein and co-workers, *J. Chem. Soc.*, **107**, 1491 (1915); **109**, 596 (1916); **117**, 975, 1598 (1920); THIS JOURNAL, **46**, 2553, 2804 (1924). With reference to hydroxyl group β see: Pechmann, *Ber.*, **27**, 1888 (1894); **28**, 857 (1895); Werner, *J. Chem. Soc.*, **115**, 1098 (1919); Biltz and Paetzold, *Ann.*, **433**, 86 (1923); Nierenstein, *Ber.*, **60**, 1820 (1927).

[5] Nierenstein, *J. Chem. Soc.*, **119**, 167 (1921); THIS JOURNAL, **48**, 1964 (1926).

[6] Nierenstein, *ibid.*, **117**, 974 (1920).

assumption suggested to the writer by Professor J. Meisenheimer of Tübingen, and Dr. W. H. Mills, F.R.S., of Cambridge, that the other stereoisomeric form is produced in such a small quantity (less than 1% as evident from several attempts to isolate it) that it escapes detection.

Experimental

The twigs used in this investigation were sent by courtesy of the authorities of the Forest Research Institute in Dehra Dun, India. They arrived in two consignments in 1921 and 1924, respectively. Each consignment was investigated separately and identical products were obtained from both, a total of 850 kilos of finely powdered young twigs being examined.

l-**Leucomaclurin-glycol Ether.**—The most striking difference between this substance and catechin is that *l*-leucomaclurin-glycol ether is very soluble in cold water, which accounts for the fact that it has so far been overlooked. For the extraction 5 liters of distilled water per kilo of powdered twigs are used and a little chloroform added to prevent infection. The pressed out and filtered extracts are decolorized with lead acetate, a little lead carbonate being added, and the filtered solution freed from lead with the aid of hydrogen sulfide. The filtrate from the lead salt is boiled, filtered when cold, and concentrated *in vacuo* to about 50 cc., when, on standing, faintly yellow-colored crystals are deposited. In this manner from 1 kilo of the powdered twigs a maximum yield of 0.5 g. of crude product is obtained. The total yield of crude material obtained from 850 kilos of powdered twigs is 182 g. For further purification 3 g. of the product is dissolved in 50 cc. of distilled water, to which a few drops of acetic acid are added, boiled with a little charcoal and the filtered solution left slowly to evaporate over concd. sulfuric acid. This process is best repeated twice; yield of pure product, 114 g.

l-**Leucomaclurin-glycol ether** (II) crystallizes in microscopic needles, which melt at 198° without previous sintering when pure, although some darkening may be observed at about 170–180°. It is easily soluble in water, alcohol, acetone and ether. The aqueous solution turns green with ferric chloride, yellow on warming with alkali and gives a deep red coloration on pine wood when tested with phloroglucinol. In this respect it differs from catechin, which gives a purplish coloration for phloroglucinol. The following rotations are given by *l*-leucomaclurin-glycol ether: $[\alpha]_D^{19}$ −62.57 (in water), $[\alpha]_D^{19}$ −41.59 (in alcohol), $[\alpha]_D^{18}$ −11.48 (in acetone).

Anal. (I) Freshly crystallized, air-dry material dried in a vacuum over phosphorus pentoxide at 100°. Subs., 13.14, 11.61 mg.: H_2O, 2.36, 2.07 mg. Calcd. for $C_{15}H_{16}O_7 + 4H_2O$: H_2O, 18.95. Found: 17.96, 17.83. (II) Material dried in desiccator over calcium chloride. Subs., 7.812, 5.924 mg.: CO_2, 14.810, 11.325 mg.; H_2O, 4.165, 3.139 mg. Calcd. for $C_{15}H_{16}O_7 + 2H_2O$: C, 52.33; H, 5.81. Found: C, 51.71, 52.15; H, 5.97, 5.93. (III) Anhydrous material, obtained by drying in a vacuum over phosphorus pentoxide at 100°. Subs., 9.54, 10.78 mg.: CO_2, 20.41, 23.08 mg.; H_2O, 4.47, 5.07 mg. Calcd. for $C_{15}H_{16}O_7$: C, 58.44, H, 5.20. Found: C, 58.35, 58.39; H, 5.24, 5.26.

Tetramethyl Ether (IV).—To a suspension of 1 g. of anhydrous *l*-leucomaclurin-glycol ether in 100 cc. of ether is added an ethereal solution of diazomethane from 8 cc. of nitrosomethylurethan. The residue (1.1 g.) left on evaporation of the ether crystallizes from alcohol in colorless pointed needles which melt at 147°. The product is soluble in the usual organic solvents, but quite insoluble in water, the alcoholic solution giving no coloration with ferric chloride. The following rotations are observed: $[\alpha]_D^{19}$ −36.40° (in alcohol), $[\alpha]_D^{16}$ −22.20° (in 50% acetic acid).

Anal. Subs., 11.26 mg.: CO_2, 25.79 mg.; H_2O, 6.81 mg. Subs., 3.21 mg.; AgI, 8.24 mg. Calcd. for $C_{19}H_{24}O_7$: C, 62.64; H, 6.59; OCH_3, 34.07. Found: C, 62.46; H, 6.77; OCH_3, 33.89.

Hexamethyl Ether (V).—The methylation both of substances (II) and (IV) is carried out by suspending the products in an excess of methyl iodide diluted with twice its volume of anhydrous ether and adding the theoretical amount of silver oxide, calculated on the methyl iodide used. Soon after the silver oxide is added, the reaction sets in and is completed by refluxing on a water-bath until the odor of methyl iodide disappears, which requires about one and one-half to two and one-half hours. The filtered solution is evaporated and the solid obtained dissolved in alcohol, filtered from a flocculent by-product and precipitated with water, when a nearly theoretical yield of the crude hexamethyl ether (V) is obtained. It crystallizes from alcohol in small needles and from benzene and ligroin (1:1) in long prismatic needles. It melts sharply at 97°. The product is soluble in all organic solvents, but quite insoluble in water. It is optically active, the following values being obtained: $[\alpha]_D^{19}$ −63.40° (in alcohol), $[\alpha]_D^{19}$ −35.99° (in acetone), $[\alpha]_D^{17}$ −74.55° (in carbon tetrachloride).

Anal. Subs., 10.06 mg.: CO_2, 23.67 mg.; H_2O, 6.53 mg. Subs., 2.76 mg.: AgI, 9.98 mg. Calcd. for $C_{21}H_{28}O_7$: C, 64.29; H, 7.14; OCH_3, 47.45. Found: C, 64.17; H, 7.26; OCH_3, 47.73.

Hydrolysis of the Hexamethyl Ether (V).—To a solution of 5 g. of substance (V) in 50 cc. of methyl alcohol is added 20 cc. of a 25% solution of sodium in methyl alcohol and the mixture refluxed for five hours. The greater part of the alcohol is removed on evaporation and the product obtained is steam distilled, the distillate being freed from alcohol by warming on the water-bath and then extracting with ether. The dried ethereal solution is evaporated to dryness, the residue dissolved in 5 cc. of dry pyridine and treated with 3 g. of triphenylchloromethane according to Helferich, Speidel and Toeldte.[7] In this manner 2.1 g. of crude methyltriphenylmethylglycol is obtained. The product crystallizes from alcohol in big needles which melt at 104°, the melting point not being depressed on admixture with authentic methyltriphenylmethylglycol.[8]

Anal. Subs., 8.68 mg.: CO_2, 26.39 mg.; H_2O, 5.49 mg. Subs., 6.22 mg.; AgI, 4.66 mg. Calcd. for $C_{22}H_{22}O_2$: C, 83.02; H, 6.92; OCH_3, 9.75. Found: C, 82.92; H, 7.08; OCH_3, 9.89.

The solution from which the methyl glycol has been removed on steam distillation is acidified and extracted with ether. The dried ethereal solution is filtered and treated with excess of an ethereal solution of diazomethane. The solid left on evaporation of the ether crystallizes from alcohol in colorless needles which melt at 103°, the melting point not being depressed on admixture with authentic hexamethyl-leucomaclurin prepared according to Kostanecki and Lampe.[9]

Anal. Subs., 7.82 mg.: CO_2, 18.76 mg.; H_2O, 4.88 mg. Subs., 2.71 mg.; AgI, 11.16 mg. Calcd. for $C_{19}H_{24}O_6$: C, 65.52; H, 6.89; OCH_3, 53.45. Found: C, 65.42; H, 6.98; OCH_3, 54.36.

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[7] Helferich, Speidel and Toeldte, *Ber.*, **56**, 766 (1923).

[8] Nierenstein, *Ber.*, **60**, 1821 (1927).

[9] Kostanecki and Lampe, *Ber.*, **39**, 4021 (1906). The melting point given by these authors is 94–96° for the product prepared by the action of veratroyl chloride on trimethylphloroglucinol, reduction of pentamethylmachurin to the leuco product and methylation of the latter. The product obtained by me melted, however, at 103°. For comparison hexamethyl-leucomaclurin was also prepared by methylation of maclurin with dimethyl sulfate as well as methyl iodide in methyl alcoholic solution. These two products also melted at 103° and gave correct analytical data.

Production of Penta-acetyl-*l*-acacatechin from *l*-Leucomaclurin-glycol Ether.—A solution of 5 g. of *l*-leucomaclurin-glycol ether in an excess of acetic anhydride is heated for forty minutes and the cold solution poured into water. The white solid formed on standing melts in the crude state at 149–151° and is practically pure penta-acetyl-*l*-acacatechin, which crystallizes from alcohol in needles melting at 151°. This melting point is not depressed when penta-acetyl-*l*-acacatechin from *l*-leucomaclurin-glycol ether is mixed in varying proportions with the penta-acetyl derivative of *l*-acacatechin from the heart-wood of *Acacia Catechu*, Willd. The rotation of penta-acetyl-*l*-acacatechin from *l*-maclurin-glycol ether in tetrachloro-ethane is $[\alpha]_D^{21}$ −12.0°, whereas penta-acetyl-*l*-acacatechin from *l*-acacatechin rotates in the same solvent $[\alpha]_D^{20}$ −11.5°, the same concentration being used in both cases.

Anal. Subs., 5.222, 4.642 mg.: CO_2, 11.376, 10.171; H_2O, 2.397, 2.084 mg. Calcd. for $C_{25}H_{24}O_{11}$: C, 60.00; H, 4.80. Found: C, 59.41, 59.76; H, 5.14, 5.02.

For the greater part of the analytical data the author is indebted to Miss Christina M. Fear. He also wishes to thank the Colston Research Committee of the University of Bristol for a grant which has covered the expenses of the investigation.

Summary

It is shown that the young twigs of *Acacia Catechu*, Willd. contain *l*-leucomaclurin-glycol ether, which on acetylation yields penta-acetyl-*l*-acacatechin, the acetyl derivative of the catechin present in the heart-wood of this tree.

BRISTOL, ENGLAND

[CONTRIBUTION FROM THE ABBOTT LABORATORIES]

5,5-SUBSTITUTED BARBITURIC ACIDS[1]

BY E. H. VOLWILER AND D. L. TABERN

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Of the many types of chemical compounds which can produce sleep, the derivatives of barbituric acid continue to be by far the most important. During recent years, the availability of new alcohols has made possible the more systematic study of the homologs of the parent member of the series, barbital (diethylbarbituric acid), with the result that several new hypnotics of superior therapeutic merit have become available. More than a hundred barbituric acid derivatives have been synthesized and most of them have been pharmacologically tested; a number of them have found their way into clinical use.

Considerable variation in sleep-producing activity exists among the members of this series, ranging from none at all to over four times that of barbital. If this increase in efficiency over barbital were merely accompanied by a corresponding increase in toxicity, there would be little attained by the study of the newer barbituric acid derivatives. This is not neces-

[1] Presented before the Medicinal Products Division of the American Chemical Society at Minneapolis, Sept. 10, 1929.