in phenacetylurea but is much less so in benzyl allophanate.

Experimental Part[6]

All alcohols used in this work were purchased from commercial sources or were prepared by published methods. None is new.

Cyanic acid was generated by subliming cyanuric acid from a 25-cc. flask connected by a glass joint to a Pyrex tube heated to dull redness in an electric combustion furnace. The exit end of the tube was bent downward to lead the effluent gases into a 50-cc. flask containing the alcohol. A gentle current of carbon dioxide was used to prevent accidental sucking back.

In a typical run, 5 g. of cyanuric acid was placed in the generator and 10–15 cc. of the alcohol in the receiver which was clamped in a pan of cold water. When less than 5 cc. of alcohol was available, 10 cc. of dry ether was used as solvent. The cyanuric acid was heated with a free flame, and the carbon dioxide was adjusted so that one bubble per second appeared. Sublimation of the bulk of the cyanuric acid required about fifteen minutes. The receiver was removed, stoppered and left to itself for twenty-four hours.[7] When the reaction was at an end as evidenced by disappearance of the acrid odor of cyanic acid, the allophanate was separated by filtration and washed with a little ether. It was purified to constant melting point by crystallization from an ethanol solution previously treated with carbon to aid in removal of a trace of cyanuric acid. Yields were usually 2–4 g. Carbamates, formed as by-products, account, in part, for the small yields.[5,8]

A single example of the potassium cyanate method is representative. To 25 g. of glacial acetic acid and 15 g. of *t*-amyl alcohol was added portion-wise with stirring 8.1 g. of potassium cyanate. The temperature rose to 50° and gradually subsided. After the mixture had stood several hours, it was diluted with water and extracted with ether. The ether was washed with water and evaporated on the steam-bath. The residue after crystallization from ethanol yielded 0.9 g. of *t*-amyl allophanate; m. p. and mixed m. p. 166–168°.

(6) Microanalyses by E. F. Shelberg and staff. Melting points are uncorrected.

(7) It should be emphasized that the reaction of cyanuric acid with alcohols is not always rapid. α-Phenylethanol, for example, required several days; simple alcohols, however, usually react so rapidly that the mixture becomes hot.

Summary

Some esters of allophanic acid have been prepared and tested for anticonvulsant activity. The most active compounds are those derived from secondary and tertiary alcohols of 5–7 carbon atoms. The allophanates are mild hypnotics.

(8) Béhal, *Bull. soc. chim.*, [4] **25**, 475 (1919).

NORTH CHICAGO, ILLINOIS RECEIVED NOVEMBER 2, 1949

[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY OF THE NATIONAL RESEARCH COUNCIL]

The Biogenesis of Alkaloids. I. The Isolation of N-Methyltyramine from Barley[1]

BY SAM KIRKWOOD AND LÉO MARION

During a study of the route of synthesis of the alkaloid hordenine (N-dimethyltyramine) in barley roots it was discovered that certain strains produce N-methyltyramine and not hordenine. N-Methyltyramine has never been isolated previously from a natural source although Winterstein produced it by the action of putrefactive bacteria on the alkaloid surinamine (N-methyltyrosine).[2]

The biogenesis of hordenine has been investigated by Raoul who attempted to demonstrate that the alkaloid arose from the decarboxylation of tyrosine coupled with a methylation of the resulting tyramine by formaldehyde. Persistent attempts to gain evidence for this route failed.[3] The isolation of N-methyltyramine indicates that the plant synthesizes this substance and hordenine by the methylation of tyramine through a mechanism similar to that known to exist in certain molds and in animals. Horowitz, Bonner and Houlahan have shown, by a study of mutants induced by ultraviolet irradiation, that the mold *Neurospora crassa* synthesizes choline by an enzymatic stepwise methylation of ethanolamine.[4] It is known that the animal can perform this same synthesis if it has available a supply of labile methyl groups.[5] There is some evidence in the literature that a similar stepwise enzymatic process is used by the plant in the N-methylation of alkaloids. Thus N-methylethanolamine and N-dimethylethanolamine, the two intermediates in the synthesis of choline by *N. crassa* and by animals, occur as esters in alkaloids.[6,7] This suggests that both these substances are produced by a stepwise methylation entirely analogous to that in *N. crassa* except that the plants concerned can stop the process at intermediate stages. Similarly in *Trichocereus candicans B.* and *R.* the alkaloids candicine (β-(4-hydroxyphenyl)-ethyltrimethylammonium hydroxide) and hordenine occur together.[8] This again suggests a stepwise methylation of tyramine, the plant being able to stop at both stages.

The following barleys have been found to produce N-methyltyramine: Montcalm, Olli, Sanalta and O.A.C. 21. All attempts to isolate hordenine from them have failed and it is presumed that they produce only N-methyltyramine. This points to a biochemical difference in these strains similar to that produced in *N. crassa* by ultraviolet

(1) Published as National Research Council Bull. No. 2131.

(2) Winterstein, *Z. physiol. Chem.*, **105**, 20 (1919).

(3) Raoul, *Ann. fermentations*, **3**, 129 (1937); **3**, 193 (1937); **3**, 385 (1937).

(4) Horowitz, Bonner and Houlahan, *J. Biol. Chem.*, **159**, 145 (1945); Horowitz, *ibid.*, **162**, 413 (1946).

(5) Stetten, *ibid.*, **140**, 143 (1941); du Vigneaud, Chandler, Simmonds, Noyer and Cohn, *ibid.*, **164**, 603 (1946).

(6) Faltis and Holzinger, *Ber.*, **72**, 1443 (1939).

(7) Blount, Openshaw and Todd, *J. Chem. Soc.*, 286 (1940).

(8) Reti, *Compt. rend. soc. biol.*, **114**, 811 (1933).

irradiation. This difference may have arisen as the result of a spontaneous mutation. In this case the character would be expected, by analogy with *Neurospora*, to be inherited as a single-gene difference. Proof that this is so will require genetic studies on these strains.

For the purpose of identifying the naturally occurring N-methyltyramine the synthesis reported by Walpole was reinvestigated and found to give poor yields.[9] Further, the properties of some of the intermediates obtained in this synthesis were found to be quite different from those on record (see Experimental part). An application of the methylation technique of Kindler and Peschke[10] to the methyl ether of tyramine resulted in the synthesis of N-methyltyramine in high yield and proved to be a much better route to this compound than that followed by Walpole. Recently, a further synthesis of this substance has been reported.[11]

Acknowledgment.—The authors wish to thank Mr. P. R. Cowan, Senior Cerealist at the Dominion Experimental Farm, Ottawa, who provided them with the barleys used in these experiments.

Experimental

Isolation of N-Methyltyramine from Barley Roots.—Barley (600 g.) was evenly divided among twelve 22 × 22 cm. glass trays. Distilled water (50 ml.) was added to each tray and they were stored in a closed germination cabinet. The barley was watered daily by spraying with an atomizer. On the tenth day the barley was harvested by clipping off the stems as close to the root as possible.[12] The root mats were then dried in an oven at 110° and the seeds and roots roughly separated by screening through a no. 7 wire mesh screen. The root fraction which passed through the screen was extracted with methanol for twenty-four hours in a Soxhlet extractor. The extract was taken to dryness under reduced pressure and the residue taken up in 2 *N* sulfuric acid (200 ml.). This solution was extracted three times with an equal volume of diethyl ether. The aqueous layer was placed in a continuous liquid-liquid extractor, made alkaline with concentrated ammonium hydroxide and extracted for forty-eight hours with diethyl ether. The ether extract was then reëxtracted with 200 ml. of 2 *N* sulfuric acid, the aqueous layer made alkaline with ammonium hydroxide and extracted for forty-eight hours with ether. The ether extract was dried over anhydrous sodium sulfate and the ether was removed under reduced pressure. The residue, which crystallized, was distilled under reduced pressure. It distilled as a colorless liquid (b. p. 130° at 0.05 mm.) which crystallized immediately in the receiver; yield, 168 mg. The product crystallized from anisole in the form of stubby needles, m. p. 130–131°[13] either alone or on admixture with an authentic sample of N-methyltyramine.

Anal. Calcd. for $C_9H_{13}ON$: C, 71.52; H, 8.61: N, 9.27. Found: C, 71.55; H, 8.60; N, 9.11.

Chloroplatinate of N-Methyltyramine.—The above material was dissolved in 5 *N* hydrochloric acid (0.5 ml.) and chloroplatinic acid (0.5 g.) was added. The chloroplatinate crystallized immediately in the form of orange platelets. The crystalline material was filtered off, washed twice with 1 ml. of 5 *N* hydrochloric acid; yield, 246 mg. (62%) of material melting at 205–206° (dec.) either alone or after admixture with an authentic sample of N-methyltyramine chloroplatinate. Winterstein[2] reports 205° (dec.).

Hordenine Methiodide.—The above chloroplatinate was decomposed by passing hydrogen sulfide into a water solution. N-Methyltyramine was recovered from the filtrate by continuous ether extraction as described above. The resulting compound which melted at 130–131° was refluxed for ten minutes with a solution of 1 ml. of methyl iodide in 5 ml. of methyl alcohol. The solution was concentrated to a small volume and upon cooling, hordenine methiodide crystallized in the form of colorless needles. It melted at 233–234°, alone and upon admixture with an authentic sample.

N-Acetyl-β-(4-methoxyphenyl)-ethylamine.—This compound was described by Walpole[9] as a colorless oil. When, however, it was prepared by the acetylation of O-methyltyramine in the presence of pyridine instead of sodium acetate, the product proved to be a beautifully crystalline solid. Recrystallization from a mixture of benzene and petroleum either yielded colorless needles, m. p. 85–86°.

Anal. Calcd. for $C_{11}H_{15}O_2N$: C, 68.39; H, 7.77. Found: C, 68.46; H, 7.71.

N-Acetyl-N-methyl-β-(4-methoxyphenyl)-ethylamine.—This substance was described by Walpole[9] as an oil which crystallized but could not be purified by recrystallization. When Walpole's synthesis was repeated using his conditions, the product deposited crystals which proved to be starting material (N-acetyl-β-(4-methoxyphenyl)-ethylamine). When the time of heating with sodium mentioned by Walpole was doubled, only the oily methylated product resulted, which consisted of a colorless oil boiling at 108° at 0.1 mm.

Anal. Calcd. for $C_{12}H_{17}O_2N$: C, 69.56; H, 8.21. Found: C, 69.61; H, 8.08.

N-Methyl-β-(4-methoxyphenyl)-ethylamine.—β-(4-Methoxyphenyl)-ethylamine[10] (30 g., 0.198 mole) was mixed with freshly distilled benzaldehyde (21 g., 0.198 mole). An immediate reaction took place and the resulting water was removed under reduced pressure. Freshly distilled dimethyl sulfate (25.4 g., 0.202 mole) in 50 ml. of dry benzene was then added and the mixture heated to 90° on a steam-bath. The solution separated into two phases in a few minutes and was kept at 90° for thirty minutes. The two phases were then separated and the lower one was washed twice with its own volume of benzene. It was then dissolved in 300 ml. of 80% ethanol and refluxed for half an hour, after which water (20 ml.) was added to the solution and the alcohol removed under reduced pressure. The remaining aqueous solution was made distinctly acid with concentrated hydrochloric acid and extracted twice with its own volume of ether. The aqueous layer was then made alkaline with concentrated potassium hydroxide solution while cooling and the liberated amine was extracted with ether. The ether extract was dried with anhydrous sodium sulfate and the ether removed under reduced pressure. The amine distilled at 127° at 9 mm.; yield, 29 g. (89%).

N-Methyltyramine.—N-Methyl-β-(4-methoxyphenyl)-ethylamine (20 g.) was refluxed with 200 ml. of 48% hydrobromic acid for three hours. The greater part of the hydrobromic acid was removed under reduced pressure and the resulting solution placed in a continuous liquid extractor, made alkaline with concentrated ammonium hydroxide and extracted for forty-eight hours. The extract was dried with anhydrous sodium sulfate and the ether removed under reduced pressure. The residue when distilled under reduced pressure (b. p. 135° at 0.05 mm.) crystallized immediately in the receiver, m. p. 130–131°; yield, 17 g. (93%). It could be recrystallized with ease from boiling anisole.

(9) Walpole, *J. Chem. Soc.*, **97**, 943 (1910).

(10) Kindler and Peschke, *Arch. Pharm.*, **270**, 340 (1932).

(11) Corti, *Helv. Chim. Acta*, **32**, 681 (1949).

(12) It is essential that the stems be largely separated from the roots before extraction. The stems of some of the strains investigated contained the alkaloid gramine (3-dimethylaminomethylindole) which reacts with N-methyltyramine in boiling methanol.

(13) All melting points reported in this paper are corrected.

Summary

1. The occurrence of N-methyltyramine in certain strains of barley is established.

2. The significance of the occurrence of this substance in relation to the problem of the mechanism of the N-methylation of alkaloids by plants is discussed.

3. An improved synthesis for N-methyltyramine is described.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY]

The Structure of Guaran[1]

BY Z. F. AHMED[2] AND ROY L. WHISTLER

Guaran,[3] the principal polysaccharide of the endosperm of guar seeds (*Cyamopsis tetragonaloba* Taub.), is a galactomannan consisting of approximately 36% D-galactose anhydride and 64% D-mannose anhydride. In the past several years, various workers have contributed information regarding the structure of this polysaccharide. Swanson[4] has shown that the galactose units occur principally as chain ends, since from the methylated polysaccharide approximately 90% of the galactose can be recovered as 2,3,4,6-tetramethyl-D-galactose. Whistler, Li and Dvonch[5] confirmed this work by finding that on periodate oxidation of guaran one mole of formic acid is produced for approximately every three anhydroglycosidic units. While measurement of the total amount of periodate consumed seemed to agree[5] with the findings of Moe, Miller and Iwen,[6] later investigations by Mr. William Dvonch of this Laboratory[7] have shown that with excess periodate 1.33 moles of oxidant are consumed for each anhydrosugar unit. These new data in conjunction with the known amount of formic acid produced[5] suggest that in the periodate oxidation of guaran each anhydrosugar unit is split once and one out of three units is split twice. That the molecules are highly anisodimensional has been indicated by physical measurements on films of guaran triacetate.[8]

Further information on the structure of guaran is now provided by a more extensive examination of the products obtained when the methylated polysaccharide is subjected to methanolysis. The resultant mixture is shown to consist of the methyl glycosides of 2,3,4,6-tetramethyl-D-galactose, 2,3,6-trimethyl-D-mannose and 2,3-dimethyl-D-mannose in approximately equal parts. Identity of the methylated cleavage fragments is based upon their conversion to established crystalline derivatives.

One possible structural arrangement for guaran which can be deduced from the isolation and characterization of its methylated sugar units is a main chain of 1,4′-linked anhydromannopyranose units with single side units of galactopyranose linked 1,6′ to half of the mannose units. Such a structure would be in agreement with other established information on guaran. This polysaccharide is, therefore, structurally similar to that of carob bean gum.[9,10]

Experimental

Methylation of Guaran.—A well-stirred solution of guaran (30 g.) in sodium hydroxide (1500 ml. of 30%) was treated with dimethyl sulfate (450 ml.) during ten hours under nitrogen and without the application of heat.

The viscous reaction mixture was then heated to 60° while dimethyl sulfate (150 ml.) and sodium hydroxide (450 ml.) were added simultaneously during five hours. After complete reaction of dimethyl sulfate, the mixture was carefully acidified with 5 *N* sulfuric acid whereupon the methylated gum precipitated as a gelatinous, white mass.

The partially methylated gum, dispersed in water, was subjected to four more methylations by simultaneous dropwise addition of dimethyl sulfate (400 ml.) and sodium hydroxide (1000 ml.) and heating the reaction mixture on a boiling water-bath at the end of each methylation.

The crude methylated gum was dissolved in a water–acetone mixture and subjected to dialysis in cellophane tubes against water. The solution was then evaporated to dryness under reduced pressure.

The methylated gum, after repeated precipitation from chloroform solution by petroleum ether, was obtained as a white glassy mass (28 g.); yield 75%. Fractional precipitation of the methylated gum from acetone solution, by the addition of petroleum ether, showed the methyl gum to be essentially homogeneous. The methoxyl content was 45.5% and the ash content 0.8%; $[\alpha]^{20}D$ + 42° in chloroform (*c*, 1).

Methanolysis of the Methylated Guaran.—The methylated guaran (15 g.) was dissolved in methanolic hydrogen chloride (300 ml. of 1.5%) and the solution refluxed for five hours on a boiling water-bath to a constant value of $[\alpha]^{20}D$ + 68°. The methanolysis solution was neutralized with silver carbonate, filtered and concentrated to a sirup (13.5 g.).

The glycosidic sirup (10.850 g.) was distilled at 0.01 mm. and 120–160° (bath. temp.), giving a water-white viscous sirup (10.325 g.) and a non-distillable residue (0.437 g.).

Fractionation of the Distilled Glycosides.—The distilled mixture of glycosides (10.300 g.) was slowly redistilled at 0.01 mm. and a bath temperature range of 107–170°. The data for the various fractions are presented in Table I. (The total loss during the distillation amounted to 0.122 g.)

(1) Paper No. 424 of the Agricultural Experiment Station, Purdue University, Lafayette, Indiana.

(2) Present address: School of Pharmacy, Faculty of Medicine, Kasre El Einy, Cairo, Egypt.

(3) E. Heyne and R. L. Whistler, THIS JOURNAL, **70**, 2249 (1948).

(4) J. Swanson, *ibid.*, **71**, 1510 (1949).

(5) R. L. Whistler, T. K. Li and W. Dvonch, *ibid.*, **70**, 3144 (1948).

(6) O. A. Moe, S. E. Miller and N. Iwen, *ibid.*, **69**, 2621 (1947).

(7) Unpublished work, following the method of Hirst, *et al.*; *cf.* ref. 5.

(8) C. L. Smart and R. L. Whistler, *J. Polymer Sci.*, **4**, 87 (1949).

(9) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948).

(10) F. Smith, THIS JOURNAL, **70**, 3249 (1948).