[Contribution from the Forest Products Laboratory and the Department of Biochemistry, University of California]

The Structure of Pinitol¹

By Arthur B. Anderson, D. L. MACDONALD AND HERMANN O. L. FISCHER²

The position of the methyl group in pinitol, a monomethyl ether of d-inositol, has been established by the preparation of a disopropylidene pinitol, and the preparation from this of a derivative of D(-)-tartaric acid.

Pinitol has long been known as a monomethyl ether of d-inositol³ having been discovered by Berthelot⁴ in the exudate of *Pinus lambertiana* Dougl. (sugar pine). Amongst other places, it has been isolated from the heartwood of five other *Haploxylon* pines (soft or white pines)⁵ and also from *Sequoia sempervirens* (redwood).⁶ The configuration of d-inositol has been shown by Posternak⁷ to be (I) but the position of the methyl group in pinitol has not been determined heretofore. Work by one of us (A.B.A.) on the extraction of pinitol from sugar pine, made available large quantities of this material, and so the present study was undertaken.

Acetonation of pinitol with acetone and hydrogen chloride under mild conditions produced a diisopropylidene derivative in good yield.⁸ Methylation of diisopropylidene pinitol with sodium and methyl iodide yielded dimethyl diisopropylidene *d*-inositol, from which the isopropylidene residues were removed by acid hydrolysis to yield a dimethyl *d*-inositol. Periodate cleavage followed by bromine oxidation yielded dimethyl-D-(-)-tartaric acid, isolated as the bis-(methylamide) of m.p. 209.5-210.5° and $[\alpha]^{24}$ D - 134° (*c* 1.62, water), in agreement with the accepted values of m.p. 205° and $[\alpha]^{17}$ D -131.8°.⁹

An examination of the *d*-inositol molecule (I) shows that a p-tartaric derivative could arise from two different pairs of adjacent carbon atoms. If the dimethyl-p-tartaric acid had arisen from the dimethyl *d*-inositol (VII), then the diisopropylidene compound from which it had been derived must have contained either two *trans*-isopropylidene residues or two 1,3-isopropylidene residues. On the other hand, if the tartaric acid had been derived from the dimethyl *d*-inositol (V) then the diisopropylidene compound from which it had been prepared (IV) could contain two *cis*-isopropylidene residues. Experience in the chemistry of carbohydrates and of inositols¹⁰ has shown that acetone

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(2) Department of Biochemistry, University of California.

(3) The prefix d refers only to the sign of rotation, and not to configuration.

(4) M. Berthelot, Compt. rend., 41, 392 (1855).

(5) G. Linstedt, Acta Chem. Scand., 5, 129 (1951).

(6) E. C. Sherrard and E. F. Kurth, Ind. Eng. Chem., 20, 722 (1928).

(7) T. Posternak, Helv. Chim. Acta, 19, 1007 (1936).

(8) While this work was in progress, word was received from Dr. S. J. Angyal, of the University of Sydney, that he had prepared a diisopropylidene pinitol having the same constants as our own; his conclusions regarding the structure of pinitol are the same as our own. See S. J. Angyal and C. G. MacDonald, J. Chem. Soc., in press.

(9) W. N. Haworth and D. I. Jones, *ibid.*, 2349 (1927). These authors prepared the compound from unnatural D-(--)-tartaric acid.
(10) H. O. L. Fischer, Ber., 54, 775 (1921); G. Dangschat and H. O. L. Fischer, Naturwissenschaften, 27, 756 (1939); G. Dangschat, *ibid.*, 30, 146 (1942); T. Posternak, Helv. Chim. Acta, 33, 350 (1950).

combines preferentially with a pair of vicinal cis-hydroxyls. In any monomethylated d-inositol, at least one such pair of hydroxyls must be present. If one assumes that this pair of hydroxyls would be acetonated first, and the mildness of the acetonation conditions adds strength to this argument, then the isolation of a D-tartaric acid derivative indicates that the dimethyl d-inositol from which it was derived must have been V. Inasmuch as the positions of the two methoxyl groups in V are equivalent, this means that pinitol has Structure II.





(11) All melting points taken in sealed capillaries with Anschütz thermometers; microanalyses by Microchemical Laboratory, University of California. hours with 300 ml. of commercial acetone containing 1.2% (w./v.) hydrogen chloride. The acid was removed by shaking with basic lead carbonate and the filtered solution was concentrated at reduced pressure. The residue was taken up in a small volume of acetone, centrifuged from a small amount of insoluble matter and concentrated, and the crystalline residue dried in vacuo over P_2O_5 and NaOH for 16 hours. Two recrystallizations from a mixture of equal volumes of n-butyl ether and heptane, with hot centrifugation each time from some insoluble matter, yielded 4.59 g. of material (72%); m.p. 104.5–106°, with prior sintering at 103.5°, $[\alpha]^{23}D - 45.4^{\circ}$ (c 1.9, U.S.P. chloroform).

Anal. Calcd. for C₁₃H₂₂O₆ (274.3): C, 56.92; H, 8.09; acetone, 42.35. Found: C, 56.81; H, 7.97; acetone, 42.5.

Hydrolysis of the diisopropylidene pinitol by refluxing for 45 minutes in 0.1 N hydrochloric acid regenerated the starting material; two recrystallizations, achieved by dissolving the compound in water and adding ethanol (nine volumes) gave a product of m.p. $185-187^{\circ}$, $[\alpha]^{25}D + 65.0^{\circ}$ (c 2.5, water) in 83% yield.

Dimethyl Diisopropylidene d-Inositol (IV) .-- Diisopropylidene pinitol (10 g.) was converted to the sodium salt with sodium sand in dry ether¹² and methylated with methyl iodide for 60 hours. After removal of the excess reagent at reduced pressure, the compound was obtained pure after three recrystallizations from *n*-pentane; further pure mate-rial was obtained from the mother liquors; total yield 8.80 g. (84%), m.p. 88–90°, $[\alpha]^{23}D - 44.4^{\circ}$ (c 1.9, U.S.P. chloroform).

Anal. Caled. for C₁₄H₂₄O₆ (288.3): C, 58.32; H, 8.39; OCH₃, 21.53. Found: C, 58.46; H, 8.38; OCH₃, 21.22.

Dimethyl d-Inositol (V) .- Six grams of dimethyl diisopropylidene d-inositol was refluxed for 45 minutes in 125 ml. of 0.1 N hydrochloric acid. The solution was then concentrated at reduced pressure and the resulting solid was recrystallized from commercial absolute ethanol to give, together with material from the mother liquor, 4.15 g. (95%), m.p. 191-193°, $[\alpha]^{23}D + 73.0^{\circ}$ (c 2, water).

Anal. Caled. for C₈H₁₆O₆ (208.2): C, 46.15; H, 7.75; OCH₃, 29.81. Found: C, 46.13; H, 7.61; OCH₃, 28.9.

(12) E. Pacsu and S. M. Trister, THIS JOURNAL, 61, 2442 (1939).

Periodate titration¹³: 21.2 mg, (0.102 millimole) and 300 mg. of sodium meta-periodate (1.4 millimoles) in 50 ml. of water; moles of periodate consumed per mole of dimethyl inositol after the stated time interval were: 0.5 hour, 2.68; 1 hour, 2.74; 2 hours, 2.84; 8.5 hours, 2.99; 24 hours, 3.03; 51.5 hours, 3.05.

The tetraacetate, which crystallized on pouring the pyri-The tetratectate, which crystallized on pointing the pyr-dine-acetic anhydride acetylation mixture into water, was recrystallized three times from *n*-butyl ether; yield 63%, m.p. 102.5-103.5°, $[\alpha]^{24}$ D -1.4° (*c* 2, U.S.P. chloroform). *Anal.* Calcd. for C₁₆H₂₄O₁₀ (376.4): C, 51.06; H, 6.43; OCH₃, 16.49. Found: C, 51.12; H, 6.41; OCH₅, 16.51.

Dimethyl-p-tartaric Acid Bis-(methylamide) (VI).—Di-methyl d-inositol (750 mg.) was dissolved in 32.6 ml. of 0.433 N periodic acid, and the solution was cooled in ice for a few minutes and then allowed to stand at room tem-For a rew minutes and then allowed to stand at room temperature for two hours after which it was neutralized to a pH of 7 with solid barium hydroxide. The precipitate was removed by filtering through Celite,¹⁴ strontium carbonate (10 g.) and bromine (6 g. stirred until dissolved) were added to the filtrate, and the mixture allowed to stand at room temperature for 18 hours. The filtered solution at room temperature for 18 hours. The filtered solution was aerated to remove bromine, acidified with two ml. of 5 N hydrochloric acid and extracted continuously with ether for 48 hours. An excess of diazomethane in ether was added to the ether extract, the solution was concentrated at reduced pressure, and taken up in 25 ml. of methanol and saturated at 0° with methylamine. After 45 hours at room temperature the solution was concentrated in vacuo, methanol was added and removed in vacuo and this process repeated two or three times to remove all excess methylamine. The residue was taken up in 100 ml. of ethyl acetate, filtered hot and, after removal of the solvent, the crystalline residue was recrystallized twice from ethyl acetate, giving 536 mg. (73%), m.p. 209.5–210.5°, $[\alpha]^{24}$ D – 134° (c, 1.62, water). For dimethyl-D-tartaric acid bis-(methyl-amide), Haworth and Jones⁹ reported m.p. 205°, $[\alpha]^{19}$ D -131.8 (c 1.61, water).

Anal. Calcd. for C₈H₁₆O₄N₂ (204.2): C, 47.04; H, 7.90; N, 13.72; OCH₃, 30.39. Found: C, 47.22; H, 7.78; N, 13.62; OCH, 30.11.

(13) E. L. Jackson, in "Organic Reactions," Vol. II, John Wiley and Sons, New York, N. Y., 1944, p. 361.

(14) A product of the Johns-Manville Company.

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The Free Amino Groups of Subtilin

By John F. Carson

Deamination of the polypeptide, subtilin, with nitrous acid, followed by hydrolysis; and microbiological assay and reaction of subtilin with dinitrofluorobenzene, followed by hydrolysis and chromatographic examination of the DNP-amino Each lysine unit has its α -amino groups of subilin are contributed by lysine and the two sulfur diamino dicarboxylic acids. Each lysine unit has its α -amino group in linkage and the ϵ -amino group free. The two sulfur diamino dicarboxylic acids cannot exist in subtilin with both amino groups free. They must exist with one amino group free or neither free.

The nature of the free amino groups of the polypeptide subtilin has been investigated by deamination with nitrous acid and by the dinitrofluoroben-zene technique of Sanger.² The problem is of particular interest in the case of subtilin because of the rapid inactivation of the antibiotic in dilute alkali accompanied by an apparent decrease in amino nitrogen (Van Slyke) in isolated products.³ The isolation of mesolanthionine⁴ and a second unidentified

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) F. Sanger, Biochem. J., 39, 507 (1945); 40, 261 (1946); 42, 287 (1948).

(3) Unpublished observations.

(4) G. Alderton and H. L. Fevold, THIS JOURNAL, 73, 463 (1951).

diamino dicarboxylic sulfur acid⁵ with the empirical formula $C_5H_8S(NH_2)_2(COOH)_2$ indicates that subtilin may contain free α -amino groups of a type not ordinarily encountered in proteins or polypeptides.

The polypeptide was treated with nitrous acid for different periods of time and the deaminated products isolated and hydrolyzed with acid. The hydrolysates were then assayed microbiologically for lysine, valine, leucine, isoleucine, glycine, phenylalanine and glutamic and aspartic acids.6

(5) G. Alderton, to be published.

(6) The presence of free amino groups of tryptophan or of alanine was not investigated by this method. No accurate analytical determination for the two sulfur acids was known.