plexes can be formed and the inhibitor constants for these inactive complexes. Since V_m is the turnover number which will ultimately be of importance in evaluating the mechanism of enzymic catalysis, the existence of this type of inhibition should be kept in mind if a substrate might be attracted to the enzyme in more than one possible arrangement.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA]

The Absolute Configuration of the *myo*-Inositol 1-Phosphates and a Confirmation of the Bornesitol Configurations[1]

BY CLINTON E. BALLOU AND LEWIS I. PIZER[2]

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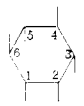
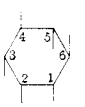
An optically active *myo*-inositol 1-phosphate and *myo*-inositol 1-O-methyl ether have been synthesized from galactinol, a galactoside of *myo*-inositol in which the absolute configuration of the 1-position of the *myo*-inositol involved in the linkage is known. Thus, the absolute configurations of the two products are known. The *myo*-inositol 1-phosphate is the enantiomorph of the product obtained by base hydrolysis of soybean phosphoinositide, while the methyl ether is identical with (+)-bornesitol, a naturally occurring 1-O-methyl *myo*-inositol. (−)-Bornesitol has been synthesized from quebrachitol (1-O-methyl-(−)-inositol) by Anderson and Post. Thus, both enantiomorphs of bornesitol have been obtained by synthesis.

myo-Inositol occurs in nature in several conjugated forms. Being a *meso*-compound, it may, like glycerol, be substituted in such a way as to form optically active conjugates. Thus, three optically active monomethyl ethers have been described[2]; the *myo*-inositol part of galactinol[3] has been shown to be asymmetrically substituted with D-galactose[4]; and, it has recently been demonstrated that the *myo*-inositol moiety in the phosphoinositide from soybean[5] and from beef liver[6] is asymmetrically linked to glycerol phosphate.

The cyclic form of the inositols has led to some difficulty in nomenclature, since there is no direct way in which positions in the inositol rings can be related stereochemically to a straight chain reference such as glyceraldehyde. However, the generally accepted convention[7] for numbering the positions in the *myo*-inositol ring leads to the two mirror images below (I and II).



I

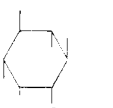


II

These differ only in the direction in which the ring is numbered, and for a substituted *myo*-inositol, that direction of numbering is followed which gives the substituent the lowest number.

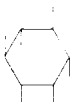
While positions 2 and 5 have planes of symmetry, positions 1,3 and 4,6 form enantiomorphic pairs. Indeed, two of the monomethyl ethers of *myo*-inositol found in nature[8] correspond to the 1,3-pair of isomers, III and IV.

III



IV

One is called (+)-bornesitol (III) and the other (−)-bornesitol (IV), the absolute configurations of the two having been established by the synthesis of the latter from quebrachitol (1-O-methyl-(−)-inositol).[9] The absolute configuration of the substituted *myo*-inositol in galactinol has been found to be (V).



Galactose

V

(−)-Inositol

VI

The D-galactose is attached to that position which on inversion leads to (−)-inositol (VI). Cleavage of the (−)-inositol ring along the dotted line would give a straight chain polyol with the L-mannitol configuration.[10]

Recent work published from this Laboratory has established that the base hydrolysis of soybean phosphoinositide yields an optically active *myo*-inositol phosphate in which the phosphate group was shown to be in one of the enantiomorphic 1-positions.[5] To establish the optical purity of this isolated material as well as its absolute configuration, we have carried out the synthesis of an asymmetric *myo*-inositol 1-phosphate. The starting material for this work was galactinol, which has the structure and absolute configuration shown in V. Complete benzylation was carried out in dimethylformamide with benzyl bromide and silver oxide according to the method of Kuhn, *et al.*[11] The

(1) Reported in part in a previous publication, C. E. Ballou and L. I. Pizer, THIS JOURNAL, **81**, 4745 (1959).

(2) S. J. Angyal, P. T. Gilham and C. G. MacDonald, *J. Chem. Soc.*, 1417 (1957).

(3) R. J. Brown and R. F. Serro, THIS JOURNAL, **75**, 1040 (1953).

(4) E. A. Kabat, D. L. MacDonald, C. E. Ballou and H. O. L. Fischer, *ibid.*, **75**, 4507 (1953).

(5) F. L. Pizer and C. E. Ballou, *ibid.*, **81**, 915 (1959).

(6) H. Brockerhoff and D. J. Hanahan, *ibid.*, **81**, 2591 (1959).

(7) H. G. Fletcher, Jr., L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

(8) A. Girard, *Compt. rend.*, **73**, 425 (1871); V. Plouvier, *ibid.*, **241**, 983 (1955).

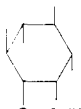
(9) L. Anderson and G. G. Post, Abstracts 134th Meeting of the American Chemical Society, Chicago, 1958, 13-D.

(10) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **75**, 3673 (1953).

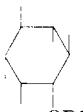
(11) R. Kuhn, I. Löw and H. Trischmann, *Ber.*, **90**, 203 (1957).

resulting nona-O-benzyl-galactinol underwent methanolysis in 1% methanolic hydrogen chloride to give a mixture of 2,3,4,5,6-penta-O-benzyl-*myo*-inositol[12] and methyl 2,3,4,6-tetra-O-benzyl-D-galactoside. This mixture was phosphorylated with diphenylphosphorochloridate. The phosphorylated product was freed of blocking groups by hydrogenolysis, and the resulting *myo*-inositol 1-phosphate was separated from the methyl D-galactoside by the use of a weak base resin.

This synthetic *myo*-inositol 1-phosphate was isolated as the crystalline cyclohexylamine salt. It showed $[\alpha]^{25}_{589}$ −3.2° (*p*H 9, water) and $[\alpha]^{25}_{589}$ + 9.3° (*p*H 2, water). The infrared spectrum and chromatographic properties were identical with the substance isolated from soybean phosphoinositide. However, since the latter showed $[\alpha]^{25}_{589}$ + 3.4° (*p*H 9, water) and $[\alpha]^{25}_{589}$ −9.8° (*p*H 2, water), it is apparent that the two are enantiomorphs. The synthetic compound VII has the absolute configuration of galactinol, and it follows that the soybean *myo*-inositol 1-phosphate is its mirror image (VIII). According to the



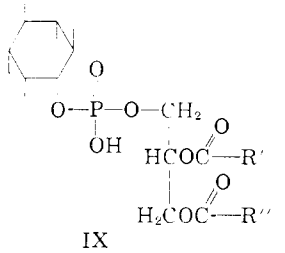
Synthetic Compound
VII



Soybean Compound
VIII

convention proposed by Lardy,[13] the synthetic compound would be called D-*myo*-inositol 1-phosphate, while the one from soybean phosphoinositide would be L-*myo*-inositol 1-phosphate.

It is now possible to assign an absolute configuration to the soybean phosphoinositide as



IX

The base hydrolysis of this lipid would lead, by way of an asymmetric 1,2-cyclic phosphate,[14] to a mixture of *myo*-inositol 2-phosphate and L-*myo*-inositol 1-phosphate. As was shown previously,[5] the latter predominates in the mixture and is readily isolated.

By use of this same general approach, we have synthesized (+)-bornesitol.[8] Methylation of the pentabenzyl *myo*-inositol, followed by removal of the benzyl groups, gave a *myo*-inositol monomethyl ether of the absolute configuration shown in III. It had $[\alpha]^{25}_{589}$ +34.0° and was in other ways identical with (+)-bornesitol which is reported to give $[\alpha]_D$ +31.4°.[15] This definitive synthesis of (+)-bornesitol confirms the structure assigned previously by Angyal, *et al.*,[2] and Anderson and Post.[9]

Angyal, *et al.*,[2] have pointed out that the only methyl ethers of *myo*-inositol found in nature have the methyl group on an equatorial hydroxyl group. This generalization may be extended to galactinol and to soybean phosphoinositide, in both of which the *myo*-inositol is substituted on an equatorial position.

Experimental

Nona-*O*-benzyl-galactinol.—Benzylation was carried out by a procedure described by Kuhn, *et al.*[11] Galactinol, 4.6 g., was dissolved in 200 ml. of dry dimethylformamide by heating. The solution was cooled to room temperature and 50 ml. of benzyl bromide and 35 g. of silver oxide were added. The mixture was shaken at room temperature for 20 hr.

The salts were filtered off by suction, and the filtrate was concentrated on a high vacuum pump at 100° bath temperature until it became thick and solid appeared to separate. The residue was extracted with four 200-ml. portions of hot benzene by heating the mixture on a steam-bath. Careful and continuous trituration of the residue with a spatula was required to ensure complete extraction. The combined benzene extracts were concentrated to a sirup on the flash evaporator, and the sirup was further concentrated in a high vacuum at a bath temperaure of 150°. A sample of this material, precipitated from hot ethanol solution by cooling, gave a carbon and hydrogen analysis that corresponded to the heptabenzyl ether. Complete benzylation was accomplished as follows.

The above sirup was dissolved in 120 ml. of benzyl chloride, and 50 g. of powdered potassium hydroxide was added. The mixture was stirred for 3 hr. at 140°, and then it was allowed to cool. Benzene (100 ml.) and water (200 ml.) were added, and the organic layer was separated and washed with water until the water washing was neutral. The organic layer was dried over sodium sulfate, the salt was filtered off and the filtrate was concentrated, first on the flash evaporator and then on a high vacuum pump at a bath temperature of 150°. The resulting sirup of impure nonabenzylgalactinol was obtained in an almost quantitative yield of 15 g. The sirup was dissolved in the minimum amount of boiling absolute ethanol, and the solution was chilled to −10°. The product was deposited as a gum, and the supernatant liquid was decanted. The gum was dried in a high vacuum at 100°. The yield was 12 g. of a brown stiff sirup that showed $[\alpha]^{25}_{589}$ +40° (*c* 1, benzene).

Anal. Calcd. for $C_{75}H_{76}O_{11}$ (1152): C, 78.0; H, 6.6. Found: C, 77.8; H, 6.8.

The infrared spectrum of this material in chloroform solution showed the complete absence of any hydroxyl absorption.

Methanolysis of Nona-*O*-benzyl-galactinol.—A mixture of 3 g. of nonabenzylgalactinol in 150 ml. of methanol containing 1.5 g. of hydrogen chloride gas was refluxed for 24 hr. At the start, the mixture was shaken vigorously every 15 minutes until the starting material had gone into solution. After 24 hr., the clear yellow solution was cooled and then concentrated to remove the methanol on a flash evaporator. The residue was taken up in benzene and water, and the organic layer was separated and washed with water until the washing was neutral. The benzene layer was dried over sodium sulfate, filtered from the salt, and the filtrate was concentrated to a dry sirup. This mixture of pentabenzyl-*myo*-inositol and methyl tetrabenzyl-D-galactoside analyzed correctly for the uptake of one methoxyl group and was obtained in almost quantitative yield (3 g.).

Anal. Calcd. for $C_{76}H_{80}O_{12}$ (1184): C, 77.0; H, 6.7; OCH_3, 2.6. Found: C, 76.5; H, 6.7; OCH_3, 2.8.

The infrared spectrum in chloroform solution showed free hydroxyl absorption at 3400 cm.$^{-1}$ almost identical in intensity to an equimolar solution of pentamethyl-*myo*-inositol.[4]

(12) According to ref. 7, this would be 1,2,4,5,6-penta-O-benzyl-*myo*-inositol, but such numbering obscures the relationship to galactinol and *myo*-inositol 1-phosphate.

(13) H. A. Lardy, in "The Vitamins," Vol. 11, edited by W. H. Sebrell, Jr. and R. S. Harris, Academic Press, Inc., New York, N. Y., 1954, p. 325.

(14) M. Faure, M. J. Morelec-Coulon and J. Le Cocq, *Compt. rend.*, **248**, 2252 (1959).

(15) A sample of (+)-bornesitol for comparison was supplied by Dr. F. E. King. F. E. King and L. Jurd, *J. Chem. Soc.*, 1192 (1953).

Phosphorylation of the Methanolysis Mixture.—To a solution of 0.90 g. of the methanolysis mixture in 2 ml. of dry pyridine was added 0.5 g. of diphenylphosphorochloridate. The reaction was left at 25° for 18 hr. A drop of water was added to the solution, and 10 minutes later it was dissolved in 25 ml. of benzene, and the organic layer was washed with water, 1 *N* hydrochloric acid, 1 *M* sodium bicarbonate and then with water. The benzene layer was dried over sodium sulfate, filtered from the salt, and the filtrate was concentrated to dryness. The yield was quantitative (1.1 g.). For analysis, the sirup was dried at 100° in the Abderhalden.

Anal. Calcd. for $C_{90}H_{93}O_{15}P$ (1418): P, 2.2; OCH_3, 2.2. Found: P, 2.3; OCH_3, 2.3.

Infrared analysis of the phosphorylated product in chloroform solution showed the absence of free hydroxyl absorption, an indication that substitution had occurred.

D-*myo*-**Inositol 1-Phosphate.**—A solution of 1.5 g. of the phosphorylation product in 100 ml. of absolute ethanol was treated with decolorizing carbon and filtered. The filtrate was shaken with hydrogen at atmosphere pressure in the presence of 3 g. of reduced and washed 5% palladium chloride-on-carbon. The hydrogen uptake was 160 ml. in 1 hr. The palladium catalyst was removed, and 1 g. of platinum oxide was added. Hydrogenolysis of the phenyl groups was then undertaken. The uptake of hydrogen was 780 ml. in 30 minutes. The platinum was removed, and 20 ml. of a dry, weak-base resin, Amberlite IR-45(OH), was added. This mixture was stirred for 6 hr. to absorb the acidic phosphate compound on the resin. The resin was filtered off and washed with water. It was then extracted batchwise twice with 25-ml. portions of water containing 3 ml. of cyclohexylamine. The extracts were combined and concentrated to dryness *in vacuo*. The residue was dissolved in 2 ml. of water, and acetone was added to cause turbidity. Crystals formed immediately and were latered filtered off and washed with acetone on the funnel. The weight, air dry, was 100 mg. The theoretical yield of di-cyclohexylammonium *myo*-inositol 1-phosphate would be 180 mg. The first crop was recrystallized in the same manner and dried in a high vacuum at room temperature for analysis.

Anal. Calcd. for $C_{18}H_{39}O_9N_2P$: N, 6.1; P, 6.8. Found: N, 5.7; P, 6.6.

The substance showed $[\alpha]^{25}_{589}$ −3.2° (*p*H 9, water) and $[\alpha]^{25}_{589}$ +9.3° (*p*H 2, water). Chromatographic analysis[5] showed one component that was indistinguishable from the optically active *myo*-inositol phosphate isolated from soybean phosphoinositide. The infrared spectra (potassium bromide pellet) of these two *myo*-inositol phosphates were also identical.

Methylation of the Methanolysis Mixture.—A solution of 1.5 g. of the methanolysis mixture in 75 ml. of dry ether was allowed to react under reflux with sodium wire for 2 hr.[16] The excess sodium was removed, and 1.0 ml. of methyl iodide was added. The mixture was refluxed for 5 hr., after which the ether solution was washed with water, dried and concentrated to dryness. The sirup weighed 1.4 g. and analyzed for the uptake of one methoxyl group.

Anal. Calcd. for $C_{77}H_{82}O_{12}$ (1196): OCH_3, 5.2. Found: OCH_3, 5.3.

The infrared spectrum in chloroform solution confirmed that the free hydroxyl group had been substituted.

D-*myo*-**Inositol 1-O-Methyl Ether.**—A solution of 1.4 g. of the above methylated product in 100 ml. of absolute ethanol was treated with decolorizing charcoal and filtered. The filtrate was shaken with hydrogen in the presence of 2 g. of reduced and washed 5% palladium chloride-on-carbon. The hydrogen uptake was very slow; therefore, the catalyst was filtered off, and a fresh 3 g. batch of catalyst was added. The hydrogen uptake was 310 ml. in 18 hr.

The catalyst was removed, and the solution was concentrated to dryness giving a sirup that weighed 300 mg. This was chromatographed on Whatman #1 paper in an acetone–water mixture (80:20).[17] The chromatogram was developed with a silver nitrate dip. [18]No galactinol (*Rf* .05) was present. Some myo-inositol (*Rf* 0.22) and methyl galactoside (*Rf* 0.61) were found, as well as a new major component (*Rf* 0.39). The total sample was separated on a cellulose column (4.5 × 60 cm.) by elution with an acetone-water (90–10) mixture in 15-ml. fractions. The new component with *Rf* 0.39 came off the column in tubes 394–460. Removal of the solvent gave 50 mg. of sirup that was crystallized from water by the addition of ethanol. The product was dried *in vacuo* at 100°. It showed m.p. 199–200°, and the m.p. of a mixture with authentic (+)-bornesitol[15] was undepressed. A water solution of the synthetic product showed $[\alpha]^{25}_{589}$ +34.0 (*c* 0.6, water).

Anal. Calcd. for $C_7H_{14}O_6$: OCH_3, 16.0. Found: OCH_3, 16.3.

The infrared spectra (potassium bromide pellet) of the synthetic D-*myo*-inositol 1-O-methyl ether and of authentic (+)-bornesitol were compared and found to be identical.

Acknowledgment.—This work was supported by grant A-884 of the United States Public Health Service.

(16) K. Freudenberg and R. Hixon, *Ber.*, **56**, 2119 (1923).

(17) C. E. Ballou and A. B. Anderson, THIS JOURNAL, **75**, 648 (1953).

(18) E. F. L. J. Anet and T. M. Reynolds, *Nature*, **174**, 930 (1954).