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Novobiocin. IX. Noviose Glycosides

BY EDWARD WALTON, JOHN O. RODIN, FREDERICK W. HOLLY, JOHN W. RICHTER, CLIFFORD H. SHUNK AND KARL FOLKERS

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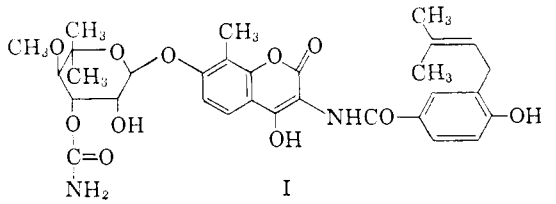
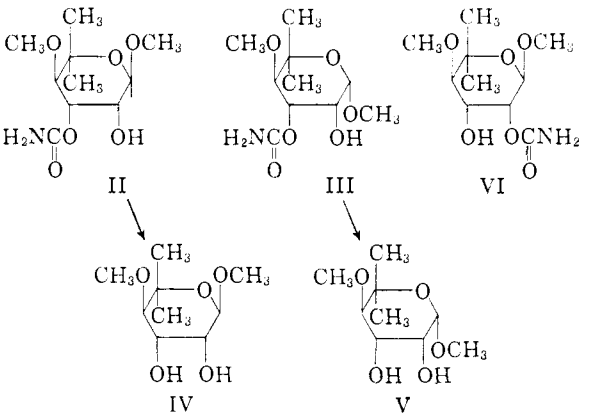
From acidic methanolysis of novobiocin, α-methyl 2-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside as well as the α- and β-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranosides have been obtained. Alkaline hydrolysis of the carbamyl group has produced the α- and β-methyl 4-O-methyl-5,5-dimethyl-L-lyxopyranosides. Optical rotational data suggest that novobiocin is an α-glycoside.

Acidic methanolysis of novobiocin (I)[1,2] yielded, in addition to a mixture of novobiocic acid and



I

cyclonovobiocic acid,[1,3] a methyl glycoside (methyl 3-O-carbamylnovioside.[1,4] This glycoside ($[\alpha]_D$ − 28°) has been shown to be methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (II).[1,5,6] As the yield of the glycoside II was rather low, it was decided to look for other products of the reaction. After a rather lengthy series of



II III VI

IV V

fractional crystallizations, two new glycosidic substances were obtained. One of these glycosides ($[\alpha]_D$ + 130°) was found to exist in two interconvertible crystalline modifications which had melting points of 117–118° and 155–157°. The elemental analyses and functional infrared spectral bands of this substance indicated that it is isomeric with methyl 3-O-carbamylnovioside (II). When the new glycoside (III) was hydrolyzed with dilute acid, it reacted with one mole of periodate. This evidence suggested that glycosides II and III were the α- and β-anomers of methyl 3-O-carbamylnovioside.

The indicated anomeric relationship was confirmed when rotations of samples of glycosides II and III reached the same equilibrium value following acidic hydrolysis to 3-O-carbamylnoviose (Fig. 1). As glycoside III has the more positive rotation, it is, conventionally, β-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside. Glycoside II, that with the negative rotation, is the α-anomer.

Having both the α- and β-methyl glycosides of 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside II and III, it is possible to apply Hudson's rules of isorotation[7] to the question of the configuration of the glycoside link in novobiocin. From the molecular rotation (−6250°) of α-methyl 3-O-carbamylnovioside (II) and that (+32,500°) of the β-anomer III, the 2B rotational value is calculated to be +26,000°. As the molecular rotation of novobiocin (−24,000°) is negative, the rotational contribution of its glycosidic carbon must be negative. This indicates that the glycoside link of novobiocin is of the α-L-configuration. On this basis, it is proposed that novobiocin is α-7-[4-hydroxy-3-(4-hydroxy-3-[3-methyl-2-butenyl]-benzamido)-8-methylcoumaryl]-3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (I).

It has been reported[1,4] that alkaline hydrolysis of α-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (II) removes the 3-O-carbamyl group producing α-methyl 4-O-methyl-5,5-dimethyl-L-lyxopyranoside (IV). Similarly, β-methyl 4-O-methyl-5,5-dimethyl-L-lyxopyranoside (V)[8] is obtained from the β-methylglycoside III.

In addition to the α- and β-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranosides (II and III), a third glycoside (VI) was obtained from the acidic methanolysis of novobiocin. The elemental analyses and functional infrared absorption bands of this compound were the same as those of the glycosides II and III. The optical rotation ($[\alpha]_D$−9.9°) precluded the possibility of this compound being a polymorphic form[9] of the glycosides II or III. Alkaline

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(2) E. A. Kaczka, C. H. Shunk, J. W. Richter, F. J. Wolf, M. M. Gasser and K. Folkers, *ibid.*, **78**, 4125 (1956).

(3) J. W. Hinman, H. Hoeksema, E. L. Caron and W. G. Jackson, *ibid.*, **78**, 1072 (1956).

(4) J. W. Hinman, E. L. Caron and H. Hoeksema, *ibid.*, **79**, 3789 (1957).

(5) E. Walton, J. O. Rodin, C. H. Stammer, F. W. Holly and K. Folkers, *ibid.*, **80**, 5168 (1958).

(6) J. W. Hinman, E. L. Caron and H. Hoeksema, *ibid.*, **78**, 2019 (1956).

(7) C. S. Hudson, *ibid.*, **31**, 66 (1909).

(8) This product has also been obtained from β-methyl 4-O-methyl-5,5-dimethyl-L-lyxopyranoside 2,3-cyclic carbonate (ref. 4). These workers have also demonstrated the anomeric relationship of glycosides IV and V.

(9) Differences in the infrared spectra (8 to 11 μ region) were not conclusive as only solid state spectra were available.

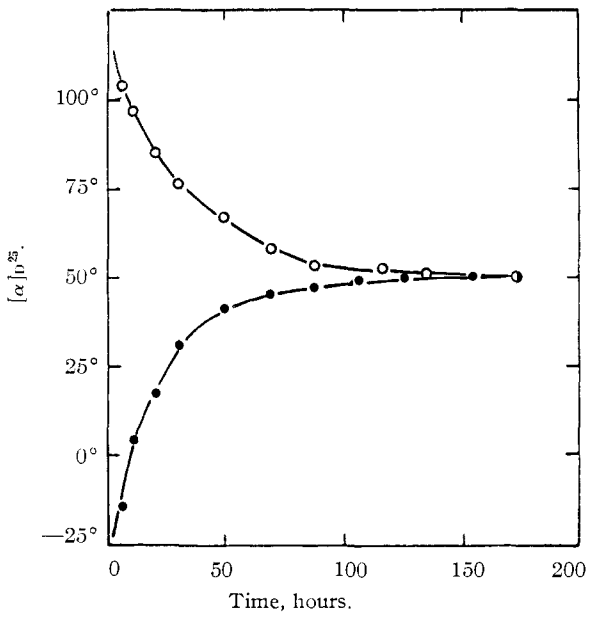


Fig. 1.—Change in rotation with time at 25° in 3 *N* hydrochloric acid: O, α-methyl 2-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (*c* 1.15); ●, β-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (*c* 2.14).

hydrolysis of compound VI to remove the carbamyl substituent gave a good yield of α-methyl 4-O-methyl-5,5-dimethyl-L-lyxopyranoside (IV). When subjected to hydrolysis with dilute acid, glyoside VI gave an aldose which failed to react with periodate which indicates that the carbamyl grouping is in the 2-position. These data lead to the formulation of this third glycoside as α-methyl 2-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (VI). This compound has been reported[10] as a hydrolysis product of iso-novobiocin. It was shown[10] that iso-novobiocin is produced by a reversible carbamyl migration when novobiocin is dissolved at *p*H 10. It is assumed that our product is the result of a similar migration occurring during an alkaline stage in the work-up of the acidic methanolysis of novobiocin.

Experimental[11]

Acidic Methanolysis of Novobiocin.—A solution of 400 g. (0.65 mole) of novobiocin, 4 l. of methanol and 43 ml. of concentrated hydrochloric acid was refluxed for two hours. Water (5.5 l.) was added and a solid precipitated. The mixture was thoroughly cooled and filtered giving 268 g. of a mixture of novobiocic and cyclonovobiocic acids, m.p. 145–165°. The filtrate was neutralized with sodium bicarbonate and concentrated to dryness under reduced pressure (the temperature was kept below 40°). The crystalline residue was triturated with six 250-ml. portions of acetone, and the acetone solution was treated with Darco G-60 and filtered. The filtrate was concentrated under reduced pressure to a volume of 500 ml. The solution was kept at 5° and 42 g. of crystalline solid (A), m.p. 190–193°, was obtained.

The mother liquor from crop A was concentrated under reduced pressure to a volume of 200 ml. and cooled, yielding 9 g. of solid in the form of white platelets (B), m.p. 208–212°. The mother liquor from B was further concentrated under reduced pressure to a volume of 100 ml. and cooled, giving 6.4 g. of a third solid (C), m.p. 130–185°, $[\alpha]^{26}D$ −22° (*c* 1 in methanol). The mother liquor from C was concentrated under reduced pressure yielding 50 g. of residual oil.

The oil was extracted with 1 l. of boiling ether, and a small amount of insoluble material was removed. One liter of ether was added to the filtrate and an additional small amount of insoluble material was separated. The ethereal solution was concentrated to a volume of 450 ml. and cooled, giving 7.6 g. of white crystalline material (D), m.p. 140–190°, $[\alpha]^{26}D$ −5° (*c* 1 in methanol). The mother liquor from crop D was concentrated under reduced pressure giving 40 g. of residual oil.

The oil was dissolved in 100 ml. of boiling chloroform and cooled giving 30 g. of white crystalline product (E), m.p. 109–116°. The mother liquor from crop E was concentrated under reduced pressure to a volume of 50 ml., and cooled giving 1.3 g. of a second crop (F), m.p. 103–155°. Concentration of the mother liquor from crop F to a volume of 25 ml. gave 2 g. of solid (G), m.p. 165–205°.

Crops C and D were combined and dissolved in hot acetone. The cooled solution gave 8 g. of product, m.p. 190–193°, which was combined with crop A giving a total of 50 g. (31%) of **α-methyl-3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside** (II).[1,5,6] Recrystallization from acetone gave a purified sample, m.p. 191–192°, $[\alpha]^{25}D$ −28° (*c* 1.0 in methanol); λ_{max}^{Nujol} 2.97, 3.04, 3.16, 5.86 and 6.15 μ, $\lambda_{shoulder}^{Nujol}$ 5.80 μ.

Combination of crops E and F gave 31.5 g. (20%) of **β-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside** (III). Recrystallization of a small sample from chloroform gave a product which melted at 117–118°. This was recrystallized giving a second crystalline modification of glycoside III, m.p. 155–157°, $[\alpha]^{26}D$ +130° (*c* 1.0 in methanol); λ_{max}^{Nujol} 2.81, 2.89, 3.05, 5.74, 5.84, 6.09 and 6.16 μ.

Anal. Calcd. for $C_{10}H_{19}NO_6$ (249.26): C, 48.17; H, 7.68; N, 5.62. Found: C, 47.99; H, 7.50; N, 5.85.

When the two forms of the β-methyl glycoside III were mixed, the mixture melted at 155–157°.

Three recrystallizations of crop B from ether gave **α-methyl 2-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (VI)**, m.p. 208–211°, $[\alpha]^{26}D$ −9.9° (*c* 1.0 in methanol).

Anal. Calcd. for $C_{10}H_{19}NO_6$ (249.26): C, 48.17; H, 7.68; N, 5.62. Found: C, 47.70; H, 7.45; N, 5.34.

Solutions of α- and β-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (II and III) in 3 *N* hydrochloric acid were kept at room temperature. The rotations of these solutions were noted from time to time, and are indicated in Fig. 1.

β-Methyl 4-O-Methyl-5,5-dimethyl-L-lyxopyranoside (V).—A solution of 0.50 g. (2.4 mmoles) of β-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (III), 2 ml. of 2.5 *N* sodium hydroxide and 25 ml. of water was heated on the steam-bath for 1.5 hr. The solution was cooled, neutralized with carbon dioxide and lyophilized. The residue was triturated with ether. The ether solution was concentrated at reduced pressure to 0.4 g. of residue which crystallized on standing. The product was recrystallized from petroleum ether (b.p. 30–60°) giving 0.28 g. (70%) of β-methyl 4-O-methyl-5,5-dimethyl-L-lyxopyranoside, m.p. 62–64°. Two recrystallizations from petroleum ether gave an analytical sample, m.p. 66–67.5°, $[\alpha]^{27}D$ +106° (*c* 0.7 in water).

Anal. Calcd. for $C_9H_{18}O_5$ (206.23): C, 52.41; H, 8.79; OCH_3, 30.1. Found: C, 52.51; H, 8.59; OCH_3, 31.0.

In a similar manner, alkaline hydrolysis of 16 g. of α-methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (II) gave 11.5 g. (87%) of α-methyl 4-O-methyl-5,5-dimethyl-L-lyxopyranoside (IV),[1,5] m.p. 69–71°, $[\alpha]^{25}D$ −50° (*c* 1.13 in water).

Alkaline Hydrolysis of α-Methyl 2-O-Carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (VI).—A solution of 1.0 g. of VI in 50 ml. of water and 4 ml. of 2.5 *N* sodium hydroxide was heated on the steam-cone for one hour. The solution was neutralized (*p*H 8) with carbon dioxide and lyophilized. The residue was triturated with four portions of ether and the ethereal extract was concentrated yielding 0.8 g. of oil. The residue was crystallized from petroleum ether. The recovery of α-methyl 4-O-methyl-5,5-

(10) J. W. Hinman, E. L. Caron and H. Hoeksema, THIS JOURNAL, **79**, 5321 (1957).

(11) The authors are indebted to Mr. R. N. Boos and associates for the microanalyses and to Dr. N. R. Trenner and Mr. R. W. Walker for the infrared spectra.

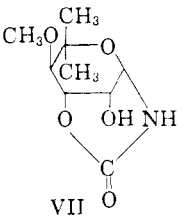
TABLE I

Compound hydrolyzed	Moles IO_4^- consumed[12] per mole cpd. 15 min.	60 min.
α-Methyl 4-O-methyl-5,5-dimethyl-L-lyxopyranoside (IV)	1.76	1.84
α-Methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (II)	0.76	0.77
β-Methyl 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (III)	0.68	0.68
α-Methyl 2-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxopyranoside (VI)	0.03	0.03

dimethyl-L-lyxopyranoside (IV), m.p. 69.5–71°, $[\alpha]^{25}D$ −45° (*c* 1.5 in water), was 0.65 g. (80%). When this product was mixed with an authentic sample of IV, the mixture melted at 69–71°.

Periodate Oxidation Studies.—Samples of the following glycosides were hydrolyzed on the steam-bath for an hour with 0.1 *N* hydrochloric acid (approximately 0.033 mmole/ml.). The hydrolysates were neutralized with equivalent amounts of 1 *N* sodium hydroxide and aliquots (containing approximately 0.1 mmole of aldose) were oxidized with excess 0.1 *M* sodium metaperiodate. The results are shown in Table I.

(12) Hinman, Caron and Hoeksema have shown (ref. 4) that acidic hydrolysis of α-methyl 3-O-carbamyl 4-O-methyl-5,5-dimethyl-L-lyxopyranoside (II) yields in addition to 3-O-carbamyl-4-O-methyl-5,5-dimethyl-L-lyxose a by-product which they have formulated as the bicyclic compound VII. Formation of such a by-product would account for the low consumption of periodate in the oxidations of II and III.



VII

RAHWAY, N. J.

[CONTRIBUTION NO. 2501 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Dissociation of Antigen–Antibody Precipitates in Alkali Chloride Solutions

BY ISIDORE S. EDELMAN[1] AND WILLIAM P. BRYAN

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The dissociation of antigen–antibody precipitates in solutions of alkali chlorides was studied by measuring the solubility of specific precipitates of purified bovine serum albumin and rabbit anti-bovine serum albumin in solutions of LiCl, NaCl, KCl and CsCl over the concentration range 0.08 to 1.6 molar. At electrolyte activities below 0.25 the solubility of the precipitate at a given activity has the order CsCl > KCl > NaCl > LiCl. Above an activity of 0.25 different results are obtained. The order of the results below an activity of 0.25 is most readily explained by the interaction of the hydrated cations with negative sites involved in the antigen–antibody bonds.

Introduction

The properties of precipitates of antigen (Ag) and antibody (Ab) have been shown to depend on the *p*H and the concentration of the inorganic salt solutions in which the Ag–Ab reaction takes place. The rate of flocculation and the amount of precipitate formed in these reactions are influenced by the *p*H and the electrolyte concentration of the medium in a variety of Ag–Ab systems, including polysaccharide:protein as well as protein:protein precipitin reactions.[2–8] In general, these experiments show that the amount of precipitate formed between antigens and rabbit antibodies is reduced at $5 > pH > 8$ and is also reduced by increasing electrolyte concentration up to 2 *M*. Furthermore, a portion of the Ag–Ab precipitate is rendered soluble by resuspension in NaCl solutions.[9,10] The evidence currently available indicates that treatment of such precipitates with saline solutions preferentially extracts antibody.[4,5,11]

Although it has been pointed out that different inorganic salts vary in their ability to reduce the amount of an Ag–Ab precipitate at equivalent ionic strength, there has been no systematic study of this effect with respect to the members of the alkali metal ions series compared at equivalent activities.[4,8] A comparison of this kind might disclose different degrees of interaction between the inorganic ions and the surfaces of the antigen and antibody molecules or, alternatively, might indicate that the dissociative effect of the electrolytes is a non-specific function of electrolyte activity. In the former case, one might expect that the smaller the ion the closer the distance of approach to the protein surface (perhaps to the specific combining site of the antigen or antibody) and the greater the dissociation of the precipitate. In the latter case, all of the univalent:univalent salts would have the same dissociative effects at the same activities and temperatures.

This paper reports the dissociative effects of the alkali chlorides (Li, Na, K, Cs) on precipitates formed from bovine serum albumin and rabbit antiserum at 3°.

(1) Senior Research Fellow in Chemistry, California Institute of Technology, on sabbatical leave-of-absence from the University of California School of Medicine, San Francisco 22, California. This study was performed during the tenure of a Special Fellowship from the National Heart Institute of the United States Public Health Service.

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(4) J. R. Marrack and H. F. Höllering, *Brit. J. Exp. Pathol.*, **19**, 424 (1938).

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(6) F. Aladjem and M. Lieberman, *J. Immunol.*, **69**, 117 (1952).

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