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Novobiocin. III. Cyclonovobiocic Acid, a Methyl Glycoside, and Other Reaction Products

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Novobiocin has a molecular formula of $C_{31}H_{36}N_2O_{11}$. Hydrogenation yielded dihydronovobiocin which is comparable with novobiocin in antibiotic properties. Cleavage of novobiocin with methanolic hydrogen chloride gave a methyl glycoside, $C_{10}H_{19}NO_6$, and cyclonovobiocic acid, $C_{22}H_{21}NO_6$. Degradation of cyclonovobiocic acid gave the known 2,2-dimethyl-6-carboxychroman. Stepwise degradation of novobiocin and dihydronovobiocin gave 4-hydroxy-3-(3-methyl-2-butenyl)-benzoic acid and 4-hydroxy-3-isopentylbenzoic acid, respectively.

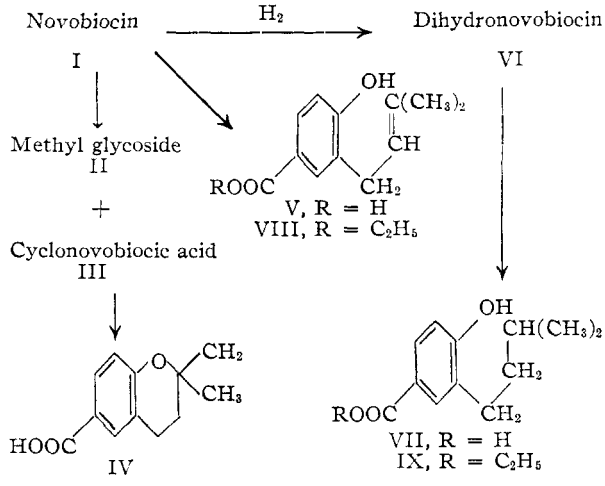
A new antibiotic designated cathomycin and streptonivicin[2] was recently reported as the result of independent investigations in two laboratories. Samples of cathomycin and streptonivicin have been exchanged[3] and were found to be identical. The same substance was also isolated in the Pfizer Laboratories and designated cardelmycin.[4] Consequently, a new generic name, novobiocin (I), has been selected for the antiobiotic. Structural studies leading to a partial structure of novobiocin have been reported.[2,5] More recently the complete structure has been elucidated.[6] In this paper are described further details on our characterization of novobiocin and on the hydrogenation and cleavage products of the molecule.

Our analytical data on novobiocin are in agreement with the formula $C_{31}H_{36}N_2O_{11}$. We previously reported[1] that the formula of novobiocin was $C_{30}H_{36}N_2O_{11}$ or a closely related one. Others[2] first reported $C_{30-32}H_{38-42}N_2O_{11}$, and then $C_{31}H_{36}N_2O_{11}$.[5]

Novobiocin was cleaved with methanolic hydrogen chloride and two compounds were characterized. One of these is a crystalline methyl glycoside (II) of a new sugar. This sugar was named 3-carbamyl-4-methylnovobiose.[6] Analytical data on the glycoside are in agreement with the formula $C_{10}H_{19}NO_6$.

Another portion of the novobiocin molecule, cyclonovobiocic acid (III), $C_{22}H_{21}NO_6$, was obtained from a methanolic hydrogen chloride cleavage reaction. Interpretation of the analytical data of novobiocin in terms of $C_{31}H_{36}N_2O_{11}$ is in agreement with the sum of the formulas of the methyl glycoside ($C_{10}H_{19}NO_6$) and cyclonovobiocic acid ($C_{22}H_{21}NO_6$) less one molecule of methanol.

Further degradation of the acid III by aqueous sodium hydroxide yielded the known acid 2,2-dimethyl-6-carboxychroman (IV).[7] 4-Hydroxy-3-(3-methyl-2-butenyl)-benzoic acid (V), the precursor of the chroman IV, was obtained by hydrolysis of novobiocin with aqueous alkali.



Hydrogenation of novobiocin over a platinum catalyst yielded dihydronovobiocin (VI). The hydrogen introduced into the molecule converted the 3-methyl-2-butenyl group into an isopentyl group. This reaction was demonstrated by cleavage of dihydronovobiocin directly with aqueous alkali to 4-hydroxy-3-isopentylbenzoic acid (VII). Biological studies with dihydronovobiocin have shown that this compound is a potent antibiotic, comparable with novobiocin itself. These antibiotic data will be reported separately.

The two acids V and VII were also prepared synthetically. Ethyl 4-hydroxy-3-(3-methyl-2-butenyl)-benzoate (VIII) was prepared by condensation of 1-bromo-3-methyl-2-butene with the sodium salt of ethyl 4-hydroxybenzoate in toluene. Hydrolysis of the ester with aqueous sodium hydroxide gave 4-hydroxy-3-(3-methyl-2-butenyl)-benzoic acid (V), which was identical with the compound obtained by degradation.

2,2-Dimethyl-6-carboxychroman (IV) was prepared from the ester VIII by cyclization with methanolic hydrogen chloride followed by alkaline hydrolysis. The chroman IV was identical with the compound obtained by degradation and with a sample kindly supplied by Dr. Walter M. Lauer.[8]

Synthesis of 4-hydroxy-3-isopentylbenzoic acid (VII) was accomplished by hydrogenation of ethyl 4-hydroxy-3-(3-methyl-2-butenyl)-benzoate (IX) over a platinum catalyst followed by alkaline hydrolysis. The synthetic acid was identical with the acid (VII) obtained from dihydronovobiocin.

(1) E. A. Kaczka, F. J. Wolf, F. P. Rathe and K. Folkers, THIS JOURNAL, **77**, 4604 (1955).

(2) H. Hoeksema, J. L. Johnson and J. W. Hinman, *ibid.*, **77**, 6710 (1955).

(3) Courtesy of Drs. R. S. Schreiber and D. I. Weisblat of the Upjohn Company.

(4) H. Welch and W. W. Wright, *Antibiotics & Chemotherapy*, **5**, 670 (1955).

(5) J. W. Hinman, H. Hoeksema, E. L. Caron and W. G. Jackson, THIS JOURNAL, **78**, 1072 (1956).

(6) C. H. Shunk, C. H. Stammer, E. A. Kaczka, E. Walton, C. F. Spencer, A. N. Wilson, J. W. Richter, F. W. Holly and K. Folkers, *ibid.*, **78**, 1770 (1956).

(7) W. M. Lauer and O. Moe, *ibid.*, **65**, 290 (1943).

(8) Department of Chemistry, University of Minnesota.

Experimental[9]

Crystalline Sodium Novobiocin.—Novobiocin was suspended in methanol (3 vol.) and neutralized to *p*H 7.5–7.6 with methanolic sodium methoxide. The solution was diluted with ten volumes of acetone and the resulting crystalline sodium novobiocin was collected. Recrystallization from hot methanol gave sodium novobiocin as glistening platelets. X-Ray diffraction studies indicated full crystallinity for the material dried at room temperature *in vacuo*, but completely dried material no longer exhibited characteristic X-ray diffraction. A second recrystallization from methanol gave sodium novobiocin of 99.8 ± 0.2% purity by solubility analysis, $[\alpha]^{25}D$ −38° (*c* 1 in water). The ultraviolet absorption spectrum of the salt in 0.1 *N* sodium hydroxide solution showed a maximum at 306 mμ ($E^{1\%}_{1cm}$ 599). The spectrum of a 0.1 *N* methanolic (90%) hydrochloric acid solution showed a maximum at 322 mμ ($E^{1\%}_{1cm}$ 387). Potentiometric titration with perchloric acid in glacial acetic acid gave an equivalent weight of 623 (calcd. 634).

Anal. Calcd. for $C_{31}H_{35}N_2O_{11}Na$: C, 58.66; H, 5.56; N, 4.42; O, 27.73; Na, 3.62. Found: C, 58.49; H, 5.51; N, 4.56; O, 27.4; Na, 3.60.

Dihydronovobiocin.—A solution of 1.12 g. of novobiocin in 20 ml. of methanol and 25 mg. of reduced platinum oxide was hydrogenated by stirring under atmospheric pressure at *ca.* 25° for 36 minutes. The hydrogen absorption was equivalent to one mole of hydrogen per mole of novobiocin. The catalyst was separated and the solution evaporated to dryness *in vacuo*. The residue of dihydronovobiocin was dissolved in acetone and precipitated from this solution with petroleum ether, $[\alpha]^{25}D$ −27° (*c* 1.68 in 2.5 *N* sodium hydroxide).

The sodium salt of dihydronovobiocin was prepared by adding one equivalent of sodium methoxide to a methanol solution of dihydronovobiocin. Recrystallization of the product from methanol gave material of 97.5% purity by solubility analysis. The ultraviolet absorption spectrum of this product in 0.1 *N* sodium hydroxide solution showed a maximum at 306 mμ ($E^{1\%}_{1cm}$ 583). The spectrum of a 0.1 *N* methanolic (90%) hydrochloric acid solution showed a maximum at 322 mμ ($E^{1\%}_{1cm}$ 381).

Anal. Calcd. for $C_{31}H_{37}N_2O_{11}Na$: C, 58.45; H, 5.86; N, 4.40; Na, 3.61. Found: C, 58.56; H, 5.74; N, 4.85; Na, 3.53.

Acid Hydrolysis of Novobiocin. A. Cyclonovobiocic Acid.—A solution of novobiocin (5.0 g.) in 250 ml. of methanol and 69 ml. of hydrochloric acid (sp. gr. 1.19) was heated on the steam-bath for two hours. After about one hour, cyclonovobiocic acid began to precipitate. The mixture was cooled and the solid was collected and washed with water; 2.7 g., m.p. 282–284°. The product was recrystallized from boiling methanol; m.p. 289–290°.

Anal. Calcd. for $C_{22}H_{21}NO_6$: C, 66.82; H, 5.35; N, 3.54. Found: C, 67.07; H, 5.21; N, 3.76.

The ultraviolet absorption spectrum of cyclonovobiocic acid in 0.1 *N* sodium hydroxide solution showed maxima at 326 mμ ($E^{1\%}_{1cm}$ 424), 290 mμ ($E^{1\%}_{1cm}$ 164) and 251 mμ ($E^{1\%}_{1cm}$ 509).

Acid Hydrolysis of Novobiocin. B. Methyl 3-Carbamyl-4-methylnovobioside.—The filtrate from the collected cyclonovobiocic acid was neutralized with sodium bicarbonate, concentrated under reduced pressure to remove the methanol, filtered, and the resulting aqueous solution was lyophilized. The residue was extracted with several portions of acetone. Concentration of the extracts to about 10 ml. gave 0.45 g. of a crystalline product, m.p. 187–190°. Recrystallization of this product from acetone raised the melting point to 191–192°, $[\alpha]^{24}D$ −24° (*c* 0.8 in methanol).

Anal. Calcd. for $C_{10}H_{19}NO_6$: C, 48.17; H, 7.61; N, 5.62. Found: C, 48.35; H, 7.54; N, 5.77.

4-Hydroxy-3-(3-methyl-2-butenyl)-benzoic Acid from Hydrolysis of Novobiocin.—A solution of 2 g. of novobiocin in 100 ml. of 30% sodium hydroxide was heated on the steam-cone for 17 hours. The dark brown solution was then acidified with dilute sulfuric acid to *ca. p*H 2. An oily precipitate (*ca.* 400 mg.) was formed, which was separated from the solution, washed with water and dried. The residue was dissolved in 10 ml. of benzene, and 2 ml. of petroleum ether (b.p. 30–60°) was added. After cooling the solution in the refrigerator, a crystalline precipitate formed; yield *ca.* 185 mg., m.p. 80–90°. The product was recrystallized from chloroform by dilution with petroleum ether; m.p. 97–99°.

The infrared spectrum of this product was identical with that of a synthetic sample of 4-hydroxy-3-(3-methyl-2-butenyl)-benzoic acid which was recrystallized from chloroform–petroleum ether. A mixed melting point determination also showed identity.

Synthesis of Ethyl 4-Hydroxy-3-(3-methyl-2-butenyl)-benzoate.—A mixture of 7 g. of sodium cut into small pieces, 300 ml. of dry toluene and 50 g. of ethyl *p*-hydroxybenzoate was heated under reflux with stirring for five hours. The mixture was cooled in an ice-bath and stirred while 50 g. of 1-bromo-3-methyl-2-butene was added dropwise during a period of two hours. The reaction mixture was stirred for 15 hours at room temperature and then warmed to 50° for one-half hour. After filtration to remove sodium bromide, the toluene solution was concentrated under reduced pressure to 100 ml. and extracted with three 100-ml. portions of 2.5 *N* sodium hydroxide. The alkaline extracts were combined, cooled to 0° and carefully acidified to *p*H 6 with 2 *N* sulfuric acid. The heavy oil which separated was extracted with ether. The ether solution was dried over magnesium sulfate and concentrated *in vacuo;* yield 42 g. This oil was dissolved in cyclohexane and extracted with a saturated solution of sodium carbonate to remove the unreacted ethyl *p*-hydroxybenzoate. The cyclohexane layer was dried over magnesium sulfate and concentrated *in vacuo*. The residue was dissolved in a boiling mixture of equal parts of cyclohexane and petroleum ether (b.p. 30–60°). After cooling at room temperature for a few hours and at 3° overnight, the solid was collected; m.p. 62–66°. Recrystallization from a mixture of cyclohexane and petroleum ether gave 18.9 g. of product, m.p. 66–69°. The analytical sample was recrystallized from cyclohexane; m.p. 70–72°.

Anal. Calcd. for $C_{14}H_{18}O_3$: C, 71.92; H, 7.74. Found: C, 71.24; H, 7.45.

Synthesis of 4-Hydroxy-3-(3-methyl-2-butenyl)-benzoic Acid.—A solution of 7 g. of ethyl 4-hydroxy-3-(3-methyl-2-butenyl)-benzoate in 30 ml. of 4 *N* sodium hydroxide was heated on the steam-cone for four hours. After cooling in an ice-bath, the solution was acidified with dilute hydrochloric acid and extracted with ether. The combined ether extracts were washed with water, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was dissolved in hot benzene and crystallized by the slow addition of cyclohexane; m.p. 80–84°. The product was recrystallized from a mixture of benzene and cyclohexane; m.p. 87–89°. Several recrystallizations from benzene raised the melting point to 94–95°. This acid was purified by dissolving it in a solution of sodium carbonate and extracting with ether. After acidifying the ice-cold sodium carbonate solution with hydrochloric acid, the product was collected and washed with cold water. The final product was recrystallized from a mixture of methanol and water; m.p. 101–103°.

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 70.28; H, 6.76.

4-Hydroxy-3-isopentylbenzoic Acid from Hydrolysis of Dihydronovobiocin.—A solution of 1 g. of dihydronovobiocin in 40 ml. of 15% sodium hydroxide was heated on the steam-cone for 18 hours. The solution was then acidified to *ca. p*H 2 with dilute sulfuric acid. The dark colored precipitate which formed was separated, washed with water, dried and then dissolved in ether. The ether solution, after treatment with Darco, was evaporated to dryness *in vacuo*, and the resulting residue was triturated with aqueous sodium bicarbonate. The precipitate which formed after acidification of the bicarbonate solution was crystallized from aqueous ethanol. The crystalline 4-hydroxy-3-isopentylbenzoic acid melted at 108–110°. Recrystallization from chloroform by dilution with ether raised the melting point to 100–102°. Other crystallizations indicated polymorphism for this acid. The infrared spectrum of this acid was identical with that of a synthetic sample of 3-isoamyl-4-hydroxybenzoic acid which had been crystallized from chloroform–ether. A mixed melting point determination also showed identity.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.20; H, 7.75. Found: C, 69.04; H, 7.70.

(9) Melting points were determined on a Kofler micro-hot-stage.

Synthesis of 4-Hydroxy-3-isopentylbenzoic Acid.—A solution of 3 g. of ethyl 4-hydroxy-3-(3-methyl-2-butenyl)-benzoate in 50 ml. of ethanol was hydrogenated over 0.5 g. of platinum oxide catalyst. The theoretical amount of hydrogen was absorbed within one hour. After removal of the catalyst by filtration, the alcohol was distilled under reduced pressure. The residue was dissolved in 20 ml. of 4 *N* sodium hydroxide and the solution was heated on the steam-cone for four hours. After acidification with hydrochloric acid, the mixture was extracted with ether. The ether extract was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was dissolved in hot benzene and cyclohexane was added. After cooling, the product was collected on a filter. The product was recrystallized from a mixture of chloroform and cyclohexane; m.p. 108–109°.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.20; H, 7.75. Found: C, 68.98; H, 7.29.

2,2-Dimethyl-6-carboxychroman from Hydrolysis of Cyclonovobiocic Acid.—A solution of 500 mg. of cyclonovobiocic acid in 20 ml. of 2.5 *N* sodium hydroxide was heated on the steam-cone for 18 hours. The dark brown solution was then acidified with dilute sulfuric acid to *ca.* *p*H 2. A dark colored crystalline precipitate formed and was separated, washed with water and dried. The crude product melted at 170–176°, with sublimation on the micro-block at *ca.* 125°. Purification was accomplished by sublimation *in vacuo* and recrystallization of the sublimate from ether by dilution with petroleum ether. The colorless 2,2-dimethyl-6-carboxychroman melted at 178–180°.

The ultraviolet absorption spectrum of the substance in solution (*ca.* *p*H 11) showed a single maximum at 252 mμ and in solution at *ca.* *p*H 2 a maximum at 262 mμ.

This compound proved to be identical with synthetic 2,2-dimethyl-6-carboxychroman and with the sample obtained from Dr. Lauer.[8]

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.90; H, 6.86; mol. wt., 206. Found: C, 69.96; H, 6.52; equiv. wt., 210.

Synthesis of 2,2-Dimethyl-6-carboxychroman.—A solution of 2 g. of ethyl 4-hydroxy-3-(3-methyl-2-butenyl)-benzoate in 15 ml. of methanol and 5 ml. of hydrochloric acid was heated under reflux for one-half hour. After concentration to dryness under reduced pressure, the residue was dissolved in a 15% aqueous solution of sodium hydroxide and the solution was heated for six hours on the steam-cone. After cooling in an ice-bath, the alkaline mixture was neutralized with 2.5 *N* hydrochloric acid. The product was collected. Recrystallization from ethanol gave colorless 2,2-dimethyl-6-carboxychroman melting at 176–178°. The melting point reported in the literature[7] is 176–177°.

The methyl ester was prepared with diazomethane in ether solution. The ester, after recrystallization from ether melted at 79–80°.

Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.88; H, 7.32. Found: C, 71.03; H, 7.60.

The *p*-bromophenacyl ester was prepared in ethanol solution from the sodium salt of the acid and *p*-bromophenacyl bromide. The ester, after recrystallization from hot ethanol, melted at 149°.

Anal. Calcd. for $C_{20}H_{19}O_4Br$: C, 59.56; H, 4.75. Found: C, 59.36; H, 4.94.

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RAHWAY, NEW JERSEY

[CONTRIBUTION NO. 971 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

The Pyridylethylation of Active Hydrogen Compounds. V. The Reaction of Ammonia, Certain Amines, Amides and Nitriles with 2- and 4-Vinylpyridine and 2-Methyl-5-vinylpyridine[1]

BY GEORGE MAGNUS AND ROBERT LEVINE

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The reactions of (1) a series of amines with 4-vinylpyridine, (2) three nitriles with 2- and 4-vinylpyridine, (3) three amines with 2-methyl-5-vinylpyridine and (4) two amides with 2- and 4-vinylpyridine are reported. The N-pyridylethylated amides, 2- and 4-$C_5H_4NCH_2CH_2NHCOR$ (R = CH_3 and C_2H_5), may be hydrolyzed to the corresponding amines, 2- and 4-$C_5H_4NCH_2CH_2NH_2$, which may also be obtained in good yields by the reactions of 2- and 4-vinylpyridine with ammonium chloride.

In previous papers from this Laboratory, the reactions of ketones[2,3] and primary[4] and secondary[5] amines with 2-vinylpyridine were discussed.

In the present paper we report the results of the reaction of (1) nine amines (two primary and seven secondary) with 4-vinylpyridine, (2) ammonia with 2- and 4-vinylpyridine, (3) three amines with 2-methyl-5-vinylpyridine, (4) two amides with 2- and 4-vinylpyridine and (5) three nitriles with 2- and 4-vinylpyridine.

The results of the additions of the amines to 4-vinylpyridine are found in Table I. It may be seen that cyclohexylamine was pyridylethylated in fair yield using a catalytic amount of acetic acid as the condensing agent. Although under these conditions or when a catalytic amount of sodium metal was used to effect the addition of aniline to 4-vinylpyridine no reaction occurred, the interaction of equivalents of aniline, 4-vinylpyridine and glacial acetic acid in methanol gave a 73.5% yield of 4-(2-anilinoethyl)-pyridine. It may be seen that the secondary amines, with the exception of pyrrole, may be condensed effectively with 4-vinylpyridine using acetic acid or hydrogen chloride as the condensing agent. Furthermore, the pseudo-acid, pyrrole, may be condensed with 4-vinylpyridine in 93% yield if sodium metal instead of an acid is used as the catalyst.

Based on previous work[4,5] there is little doubt that the products obtained from the reactions of 4-vinylpyridine with amines are derivatives of 4-(2-aminoethyl)-pyridine, *i.e.*, 4-$C_5H_4NCH_2CH_2NH_2$. However, to settle this point definitely the structures of two of the products, *i.e.*, the adducts

(1) This work was performed under Contract No. AT(30-1)-670 between the U. S. Atomic Energy Commission and the University of Pittsburgh.

(2) R. Levine and M. H. Wilt, THIS JOURNAL, **74**, 342 (1952).

(3) M. H. Wilt and R. Levine, *ibid.*, **75**, 1368 (1953).

(4) H. E. Reich and R. Levine, *ibid.*, **77**, 5434 (1955).

(5) H. E. Reich and R. Levine, *ibid.*, **77**, 4913 (1955).