reported as a degradation product of neomycin A. Our previously proposed empirical formula[1] of $C_6H_{12-14}N_2O_3$ for neamine appeared, from the molecular weight data, to represent the molecular formula. Unless the cyclohexane degradation product arises merely from racemization or rearrangement, the molecular weight data are anomalous and the molecular formula of neamine would now appear to be a multiple of C_6.

Experimental

Crystalline Neomycin A.—A 15.9-mg. sample of neomycin A hydrochloride[3] was suspended in 1 ml. of commercial methanol and the mixture was saturated with ammonia gas. The neomycin A hydrochloride dissolved completely in the ammoniacal methanol and after standing at room temperature for thirty minutes, neomycin A free base crystallized. The crystals were collected on a filter stick and washed twice with 0.5-ml. portions of methanol. The dried crystals weighed 8.0 mg. The compound decomposed in a capillary tube at 256° and showed no depression in the decomposition point when mixed with neamine.

Neamine Hydrochloride.—A 100-mg. sample of crystalline neamine prepared as described previously[1] was dissolved in 10 ml. of water and titrated to *p*H 4.5 with *N* hydrochloric acid. The solution was freeze-dried to give a quantitative yield of neamine hydrochloride.

Hydrolysis of Neamine with 48% Hydrobromic Acid.—A 5.0-g. sample of neamine was dissolved in 150 ml. of 48% hydrobromic acid and heated under reflux for 18 hours. The reaction mixture became colored rather quickly. The solution was evaporated *in vacuo* to dryness, 50 ml. of water was added and again evaporated to dryness. This process was repeated twice to insure complete removal of the excess hydrobromic acid. The residue was treated with 50 ml. of boiling methanol and filtered. The methanol insoluble fraction weighed 4.59 g. It was dissolved in 50 ml. of water, treated with 10 g. of Darco G-60, filtered and the solution concentrated *in vacuo* until crystals appeared. After refrigerating overnight, the crystals were collected, washed with 0.5 ml. of ice water and dried to yield approximately 2.5 g. of crystals. These crystals decompose at 280° (micro-block) and show no optical activity.

Anal. Calcd. for $C_6H_{14}N_2O_3 \cdot 2HBr$: C, 22.24; H, 4.98; N, 8.65; Br, 49.33, eq. wt., 162. Found: C, 22.58; H, 4.95; N, 8.64; Br, 48.58, eq. wt., 156.

The analytical data for this product are in good agreement with those calculated for the dihydrobromide of 1,3-diamino-4,5,6-trihydroxycyclohexane which has been reported by Kuehl, *et al.*,[5] to be a degradation product of neomycin A.

The methanolic extract of the hydrolysate above yielded a small amount of ammonium bromide and other unidentified degradation products.

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Preparation of Anhydrous Alcohol

BY HAKON LUND

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The method of Lund and Bjerrum[1] for the preparation of absolute alcohol by means of magnesium seems now to be in general use and is described in several books on organic syntheses[2] in the original form using iodine as a catalyst for the initiation of the reaction. It might be useful to point out that small amounts of aliphatic halogen compounds are better catalysts than iodine.[3] If traces of the halogen compound are harmless in the alcohol obtained, chloroform or carbon tetrachloride may serve, but when halogen compounds have to be strictly excluded ethyl bromide can be used. In that case the catalyst is removed with the first few cc. of the distillate.

AARHUS, DENMARK

(1) Lund and Bjerrum *Ber.*, **64**, 210 (1931).

(2) For instance L. F. Fieser, "Experiments in Organic Chemistry," and David A. Shirley, "Preparation of Organic Intermediates."

(3) *Ber.*, **37**, 936 (1934).

Reaction of Vanillin and Its Derived Compounds. XV.[1] 3-Ethoxy-4-hydroxybenzoic Acid and Some of Its Esters.[2]

BY IRWIN A. PEARL AND DONALD L. BEYER

RECEIVED JANUARY 16, 1952

The treatment of disseminated histoplasmosis with ethyl vanillate has been reported recently by Christie, Middleton, Peterson, and McVicker.[3] These investigators found that ethyl vanillate is the only known effective therapeutic agent for disseminated and progressive histoplasmosis, but that the margin between effective therapeutic levels and those which produce toxic manifestations is only about 25 to 30%, a margin of safety too small for a desirable therapeutic agent. These results led to the investigation of the effect of changes in the ethyl vanillate molecule on the therapeutic activity of the compound. The present paper reports the preparation of the related 3-ethoxy-4-hydroxybenzoic acid and representative esters prepared therefrom.

Larsson[4] has recently prepared 3-ethoxy-4-hydroxybenzoic acid from the corresponding aldehyde by a number of different procedures. We have now prepared it by the oxidation of 3-ethoxy-4-hydroxybenzaldehyde with silver oxide in aqueous alkaline solution. The low temperature caustic fusion procedure used so successfully for the preparation of vanillic acid from vanillin[5] when applied to ethylvanillin yielded only protocatechuic acid and unchanged ethylvanillin indicating that, under the conditions of caustic fusion, the ethoxyl group of ethylvanillin is more susceptible to dealkylation than is the aldehyde group to oxidation.

The desire to derive 3-ethoxy-4-hydroxybenzoic acid from our basic raw material, vanillin, led to a study of its preparation from protocatechuic acid, a compound easily prepared by caustic fusion of vanillin at temperatures above 240°.[5] Following the procedure employed by Bertram[6] for the preparation of vanillin from protocatechualdehyde, ethyl protocatechuate was treated with one mole of ethyl bromide and two moles of potassium carbonate in boiling ethanol. In addition to the desired ethyl 3-ethoxy-4-hydroxybenzoate, chromatographic separation of the reaction product yielded the ethyl

(1) For paper XIV of this series, see THIS JOURNAL, **74**, 1357 (1952).

(2) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results.

(3) A. Christie, J. G. Middleton, J. C. Peterson and D. L. McVicker, *Pediatrics*, **7**, 7 (1951).

(4) E. Larsson, *Trans. Chalmers Univ. Technol. Gothenberg*, No. **59**, 21 (1947).

(5) I. A. Pearl, THIS JOURNAL, **68**, 2180 (1946).

(6) J. Bertram, German Patent 63,007 (Aug. 19, 1890); *Ber.*, **25**, R823 (1892).

TABLE I

ESTERS OF 3-ETHOXY-4-HYDROXYBENZOIC ACID

Ester	Yield,[a] %	B.p. °C.	B.p. Mm.	M.p.,[b] °C.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Inhibiting concn., % *Bacillus mycoides*
Ethyl	92	142	2.0	64	$C_{11}H_{14}O_4$	62.84	62.83	6.71	6.69	0.09
Propyl	93	138	2.0	75–76	$C_{12}H_{16}O_4$	64.27	64.41	7.19	7.27	> .21
Isobutyl	80	151	2.5	93	$C_{13}H_{18}O_4$	65.53	65.68	7.61	7.72	.09
s-Butyl	78	139	0.6	61–62	$C_{13}H_{18}O_4$	65.53	65.51	7.61	7.63	> .21
Amyl	88	157	1.1	42	$C_{14}H_{20}O_4$	66.64	66.58	7.99	7.97	.009
Octyl	74	182	1.5	32–33	$C_{17}H_{28}O_4$	69.36	69.39	8.90	8.89	.0009
Decyl	75	197	1.5	35–36	$C_{19}H_{30}O_4$	70.77	70.67	9.38	9.21	.15

[a] Yields are of purified products. [b] All esters except octyl and decyl were recrystallized from petroleum ether (b.p. 65–110°); octyl and decyl esters recrystallized from petroleum ether (b.p. 30–60°).

esters of 4-ethoxy-3-hydroxybenzoic acid and 3,4-diethoxybenzoic acid.

The ethyl, propyl, isobutyl, *s*-butyl, amyl, octyl and decyl esters of 3-ethoxy-4-hydroxybenzoic acid were prepared by procedures employed for the preparation of analogous vanillic acid esters.[7,8] Data for these esters are given in Table I. The ultraviolet absorption spectra of 3-ethoxy-4-hydroxybenzoic acid and its esters were determined in purified 95% ethanol with a Beckman spectrophotometer. The absorption spectra for all the esters are the same as that for the acid except for actual extinction values. They are all characterized by peaks at 293, 263 and 222 mμ and are almost identical with the spectra of the corresponding esters of vanillic acid.

The inhibiting concentrations of these esters were determined for four representative aerobic microorganisms—namely, non-sporeforming (*Aerobacter aerogenes*) and sporeforming (*Bacillus mycoides*) bacteria and two molds (*Aspergillus niger* and *Penicillium expansum*). Because of the insolubility of these compounds in dilute aqueous solutions containing one equivalent of sodium hydroxide, it was necessary to employ 80% ethanol as a solvent. Except for ethyl 3-ethoxy-4-hydroxybenzoate, none of the esters of 3-ethoxy-4-hydroxybenzoic acid inhibited *Aerobacter aerogenes* or *Aspergillus niger* at concentrations as high as 0.21%. The ethyl ester inhibited these two organisms at 0.09%. *Penicillium expansum* was inhibited by the ethyl ester at 0.09%, the propyl ester at 0.15%, and the *s*-butyl ester at 0.21%. The other esters were ineffective at concentrations as high as 0.21%. The inhibiting concentrations for *Bacillus mycoides* are given in Table I. It is noteworthy that octyl 3-ethoxy-4-hydroxybenzoate is more toxic toward *Bacillus mycoides* than any ester tested to date in our studies on the esters of vanillic acid and related acids.

Experimental

All melting points are uncorrected.

3-Ethoxy-4-hydroxybenzoic Acid.—Ethylvanillin (Monsanto) was oxidized with silver oxide and alkali by the procedure reported earlier for the preparation of vanillic acid,[9] but the silver oxide and alkali ratios were doubled. The yield was quantitative. The ratios employed for vanillin gave only 50–60% yields with ethylvanillin. This was also true when only the silver oxide ratio was doubled. Silver oxide recovered by the permanganate procedure[9] was found to be inoperative in this reaction with ethylvanillin.

Lower Alkyl Esters.—All the esters except the octyl and decyl esters were prepared by boiling under reflux a mixture of 3-ethoxy-4-hydroxybenzoic acid in a fivefold excess of the esterifying alcohol for five hours, removing most of the excess alcohol under reduced pressure, diluting with water, neutralizing with sodium bicarbonate, and extracting with ether. The ether was dried and distilled to leave the crude ester which was then distilled under reduced pressure.

Higher Alkyl Esters.—The octyl and decyl esters were prepared by the iminoether synthesis employed in the past for long chain alkyl vanillates.[8]

3-Ethoxy-4-hydroxybenzonitrile.—A mixture of 156 g. of 4-acetoxy-3-ethoxybenzonitrile[4] and 450 cc. of concentrated hydrochloric acid was heated at 60–70° until all the solid dissolved. The solution was cooled, diluted with 3000 ml. of cold water, and neutralized to *p*H 4–5 with dilute sodium hydroxide. The mixture was extracted with ether, and the ether was dried and distilled to dryness. The crude 3-ethoxy-4-hydroxybenzonitrile melted at 66° and weighed 93 g. (75%). Recrystallization from petroleum ether (b.p. 65–110°) yielded white needles melting at 66°.

Anal. Calcd. for $C_9H_9O_2N$: C, 66.24; H, 5.56. Found: C, 66.39; H, 5.74.

Octyl 3-Ethoxy-4-hydroxybenzimidate Hydrochloride.—A mixture of 50 g. of 3-ethoxy-4-hydroxybenzonitrile, 41 g. of octyl alcohol and 150 cc. of absolute ether was treated with anhydrous hydrogen chloride for one hour while cooling in an ice-bath. The flask was stoppered with a calcium chloride tube and allowed to stand at 20° for 24 hours. The heavy precipitate was filtered, washed with ether, and air dried to yield 69 g. (69%) of crude octyl 3-ethoxy-4-hydroxybenzimidate hydrochloride melting at 154–155°. Purification by solution in chloroform and precipitation with ether yielded tiny white crystals melting at 155°.

Anal. Calcd. for $C_{17}H_{28}O_3NCl$: C, 61.90; H, 8.56; N, 4.25. Found: C, 61.86; H, 8.60; N, 4.45.

Decyl 3-Ethoxy-4-hydroxybenzimidate Hydrochloride.—This compound was prepared in an identical manner and was obtained in 81% yield as white crystals melting at 155–156°.

Anal. Calcd. for $C_{19}H_{32}O_3NCl$: C, 63.76; H, 9.01; N, 3.91. Found: C, 63.82; H, 9.02; N, 4.11.

Ethylation of Ethyl Protocatechuate.—A mixture of 18.2 g. (0.1 mole) of ethyl protocatechuate, 11 g. (0.1 mole) of ethyl bromide, 27.6 g. (0.2 mole) of anhydrous potassium carbonate and 100 cc. of absolute ethanol was boiled under reflux for 7 hours. The ethanol was removed under reduced pressure and was finally replaced with water. The resulting aqueous solution was extracted with ether. The ether was dried and distilled to yield 16.35 g. of viscous oil. The aqueous solution was acidified with dilute sulfuric acid and extracted with ether to yield 4.0 g. of product which, upon recrystallization from petroleum ether (b.p. 65–110°), yielded crystals of ethyl protocatechuate melting at 126–127°.

The viscous oil (2.0 g.) was chromatographed from petroleum ether (b.p. 65–110°) on a column (44 mm. in diameter and 240 mm. long) of acid washed Magnesol[10] and developed with 375 cc. of 50:1 petroleum ether–ethanol. Three bands were indicated by streaking with alkaline permanganate.[10] The leading band on elution with acetone yielded a product which, on crystallization from petroleum ether, gave colorless needles of ethyl 3,4-diethoxybenzoate melting at 52–53°.

(7) I. A. Pearl and J. F. McCoy, THIS JOURNAL, **69**, 3071 (1947).
(8) I. A. Pearl and D. L. Beyer, *ibid.*, **73**, 4091 (1951).
(9) I. A. Pearl, *ibid.*, **68**, 429 (1946).
(10) I. A. Pearl and E. E. Dickey, *ibid.*, **73**, 863 (1951).

Anal. Calcd. for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.38; H, 7.59.

Hydrolysis with ethanolic sodium hydroxide yielded 3,4-diethoxybenzoic acid which, upon recrystallization from water, was obtained as fluffy white needles melting at 162–163°.

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.74; H, 6.71.

Herzig[11] treated the tetraethyl ether of quercitin with alcoholic potassium hydroxide and obtained 3,4-diethoxybenzoic acid melting at 165–166°. He prepared the ethyl ester and recorded a melting point of 56–57°.

The other two chromatographic bands were combined and eluted. The acetone eluate, upon removal of solvent, yielded crystals and oil. The crystals were removed and recrystallized from petroleum ether (b.p. 65–110°) to give ethyl 4-ethoxy-3-hydroxybenzoate as slightly yellow crystals melting at 77–78° and not depressing the melting point of a mixture with ethyl 4-ethoxy-3-hydroxybenzoate prepared from authentic 4-ethoxy-3-hydroxybenzoic acid.[12]

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.89; H, 6.79.

The oil removed from the crystals of ethyl 4-ethoxy-3-hydroxybenzoate was boiled with dilute sodium hydroxide solution, cooled and acidified with dilute sulfuric acid. The solid obtained was filtered, washed with water, and recrystallized from dilute methanol to yield 3-ethoxy-4-hydroxybenzoic acid as white needles melting at 164–165° and not depressing a mixed melting point with authentic 3-ethoxy-4-hydroxybenzoic acid.

The approximate yields obtained in this experiment were: ethyl 4-ethoxy-3-hydroxybenzoate, 15%; ethyl 3,4-diethoxybenzoate, 20%; and 3-ethoxy-4-hydroxybenzoic acid, 30%.

Acknowledgment.—The authors wish to thank Mr. Donald McDonnell for the analyses and Mr. John Carlson for the microbiological data reported in this paper.

(11) J. Herzig, *Monatsh.*, **5**, 81 (1884).

(12) H. King, *J. Chem. Soc.*, 1157 (1939).

APPLETON, WIS.

Reductions of *i*-Cholestan-6-one[1]

BY FRANKLIN S. PROUT AND BYRON RIEGEL

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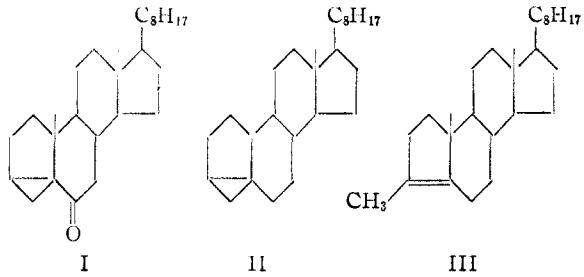
We have confirmed the preparation of *i*-cholestane (II) by the Wolff–Kishner reduction of *i*-cholestan-6-one (I)[2] recently reported by Schmid and Kagi,[3] although we have used the Huang-Minlon[4] modification of this reaction. The properties of this hydrocarbon agree with those of *i*-cholestane reported by Schmid and Kagi[3] and by Schmid and Karrer who prepared this hydrocarbon by action of lithium aluminum hydride on cholesteryl *p*-toluenesulfonate.[5]

i-Cholestane (II) failed to add hydrogen under atmospheric pressure using Raney nickel (W-4)[6] in dioxane or prereduced platinum oxide in glacial acetic acid. In both cases *i*-cholestane was recovered in 88% yield by direct crystallization of the hydrocarbon. In contrast hydrogenation of *i*-cholestane in the presence of platinum oxide and acetic acid by Schmid and Kagi[3] was followed by



I II III

treatment of the hydrocarbon with cold concentrated sulfuric acid (−10°) to give a hydrocarbon having a m.p. 43.5–44.5°, $[\alpha]^{15}D$ + 54.4° (chloroform). This compound actually appears to be an impure rearrangement product of *i*-cholestane, since *i*-cholestane was converted to compound III by shaking with concentrated sulfuric acid at 0–10°.

i-Cholestane (II) when treated with bromine according to the directions of Hauptmann[7] absorbed some bromine. The absorption was apparently random and incomplete since the only product obtained was a small amount (36%) of starting material.

Using hydrobromic acid in acetone,[8] we have confirmed the acid-catalyzed rearrangement of *i*-cholestane (II) described by Schmid and Kagi.[3] The resulting hydrocarbon (III), brilliantly characterized by these workers,[3] has been further characterized by its reaction with bromine.[7] Here the addition of bromine was accompanied by liberation of hydrogen bromide and furnished an allylic monobromide not obtained by Schmid and Kagi.[3]

In another sequence *i*-cholestan-6-one was reduced quantitatively with aluminum isopropoxide to give the *i*-cholestan-6-ol as an oil.[9] This oil was converted to cholesteryl acetate in boiling acetic acid with zinc acetate in an over-all yield of 94%.

Acknowledgment.—The authors wish to express their appreciation for a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council for the support of part of this work.

Experimental[10]

The Conversion of *i*-Cholestan-6-one (I) to Cholesteryl Acetate.—One gram (2.62 mmoles) of *i*-cholestan-6-one[11] was converted quantitatively to *i*-cholestan-6-ol by reduction with aluminum isopropoxide and isopropyl alcohol.[9] Half of this crude oil (1.27 mmoles) was heated under reflux in a mixture of 25 cc. of acetic acid, 1 g. of zinc acetate dihydrate and 1 cc. of acetic anhydride for six hours.[12] The mixture was diluted with water to give 511 mg. (94%) of

(1) Presented before the Organic Division of the American Chemical Society, 115th Meeting, March 27 to April 1, 1949, San Francisco, California.

(2) O. Windaus and A. Dalmer, *Ber.*, **52B**, 162 (1919).

(3) H. Schmid and K. Kagi, *Helv. Chim. Acta*, **33**, 1582 (1950).

(4) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946).

(5) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949).

(6) A. A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

(7) *Cf.* H. Hauptmann, *ibid.*, **69**, 562 (1947).

(8) *Cf.* B. Riegel, G. P. Hager and B. L. Zenitz, *ibid.*, **68**, 2562 (1946).

(9) I. M. Heilbron, J. Hodges and F. S. Spring, *J. Chem. Soc.*, 759 (1938).

(10) All melting points are uncorrected, except for one. The analyses were performed by Misses Margaret Hines and Virginia Gibbs of Northwestern University and by Micro-Tech Laboratories, Skokie, Illinois. Analyses for carbon and hydrogen content of *i*-cholestane (II), Compound III and the oxide of III were determined but are not reported.

(11) The *i*-cholestan-6-one (m.p. 97–98°) was prepared for us by Drs. Frank A. Vingiello and William L. Hartop according to the procedure of Windaus and Dalmer (Ref. 2).

(12) *Cf.* J. H. Beynon, I. M. Heilbron and F. S. Spring, *J. Chem. Soc.*, 406 (1937).