Anal. Calcd. for $C_{29}H_{42}O_2$: C, 80.65; H, 10.01. Found: C, 80.82; H, 10.22.

This material gave an acetate which was obtained from acetone–hexane as crystals, m.p. 184–186°; $[\alpha]D -72°$; λ_{max} 232, 239, and 247 mμ (ε 24,800, 27,200, 17,400); λ_{max} 5.75, 5.88, 6.08, 8.10, and 9.91 μ.

6-Chloro-3β-hydroxy-17-ethylpregna-4,6-dien-20-one (VIII) was recrystallized from acetone-hexane to give crystals, m.p. 192–193°; $[\alpha]D -60°$; λ_{max} 237, 244, and 252 mμ (ε 18,600, 21,700, 14,500); λ_{max} 2.89, 5.93, 6.23, and 9.72 μ.

Anal. Calcd. for $C_{29}H_{39}ClO_2$: C, 73.27; H, 8.82; Cl, 9.41. Found: C, 73.09; H, 8.80; Cl, 9.85.

17-Ethylpregna-3,5-dien-20-one (V).—A solution of crude 17-ethyl-3β-hydroxypregn-4-en-20-one (derived from 500 mg. of 17-ethylprogesterone) in 100 ml. of 50% acetic acid was heated at reflux temperature for 45 min. After 10 min. a solid was deposited from the solution. The chilled mixture was filtered to give 360 mg. of white crystals, m.p. 155–158°. This solid was dissolved in benzene and chromatographed on silica gel. The material eluted by benzene was recrystallized from methanol to give 222 mg. (47%) of white needles, m.p. 160–162°; $[\alpha]D -150°$; λ_{max} 228, 234, and 243 mμ (ε 20,200, 21,600, 13,700); λ_{max} 5.91 and 6.05 μ.

Anal. Calcd. for $C_{23}H_{34}O$: C, 84.60; H, 10.50. Found: C, 84.37; H, 10.61

Acknowledgment.—We thank Mr. W. Fulmor and his associates for the spectral and polarimetric data, Mr. L. Brancone and staff for the microanalyses, Dr. J. L. Fedrick and Mr. R. B. Conrow for a generous supply of 17-ethylprogesterone, and Dr. I. Ringler for assistance in obtaining the biological assays.

New Compounds

Derivatives of 2-Hydroxy-1,3,2-benzodioxastibole[1a]

L. CORTÉS,[1b] J. MANTECÓN,[1b] AND G. CHUCHANI

Department of Chemistry, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela

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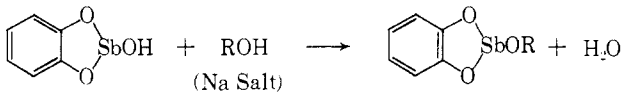
A number of derivatives of 2-hydroxy-1,3,2-benzodioxastibole (I) were synthesized as compounds of potential interest in the chemotherapy of several parasitical diseases. Several phenolic compounds containing $-COOH$ and $-SO_3H$ groups were treated with $I^{2,3}$ in basic medium to produce the corresponding condensation products. These were isolated as the sodium salts.

TABLE I

1,3,2-BENZODIOXASTIBOLE DERIVATIVES OBTAINED ACCORDING TO THE REACTION



ROH	%	Formula products[a]	% Sb Found	% Sb Calcd.
5-Chlorosalicylic acid	43	$C_{13}H_7ClNaOSb$	28.8	28.8
5-Bromosalicylic acid	39	$C_{13}H_7BrNaO_5Sb$	26.1	26.0
5-Aminosalicylic acid	39	$C_{13}H_9NNaO_5Sb$	30.2	30.1
5-Sulfosalicylic acid	26	$C_{13}H_7Na_2O_8SSb$	25.0	24.8
3-Phenylsalicylic acid	46	$C_{19}H_{12}NaO_5Sb$	26.3	26.2
3-Methylsalicylic acid	37	$C_{14}H_{10}NaO_5Sb$	30.4	30.2
6-Amino-1-naphthol-8-sulfonic acid	40	$C_{16}H_{11}NNaO_6SSb$	24.9	24.8
5-Amino-4-hydroxybenzenesulfonic acid	45	$C_{12}H_9NNaO_6SSb$	27.8	27.7
7-Amino-1-naphthol-3-sulfonic acid	36	$C_{16}H_{11}NNaO_6SSb$	24.9	24.8
8-Amino-1-naphthol-3,6-disulfonic acid	33	$C_{16}H_{10}NNa_2O_9S_2Sb$	20.5	20.6

[a] None of the compounds melts or decomposes below 300°. On acidification with hydrochloric acid, these compounds are rapidly hydrolyzed.

Experimental

2-Hydroxy-1,3,2-benzodioxastibole (I) was prepared as described by Brown and Austin.[3] The derivatives of I were prepared as described,[2] but with the following modification. After the reaction period the solid by-product (hydrated antimony oxide) was filtered and the filtrate neutralized to precipitate the unchanged I. The solution was then concentrated to incipient crystallization and the product washed with small amounts of cold ethanol.

Alternate Method of Condensation.—2-Hydroxy-1,3,2-benzodioxastibole (I) (0.03 mole) in 0.4 *N* sodium hydroxide solution was added to salicylic acid (0.035 mole) in 2 *N* sodium carbonate solution (18 ml.). The mixture was heated for 2 hr. at 70–75° and neutralized after cooling. The precipitated, unchanged I was removed by filtration and the filtrate concentrated until precipitation started. The solid, 2-(o-carboxyphenyloxy)-1,3,2-benzodioxastibole, was unchanged at 300°; yield, 70%.

Anal. Calcd. for $C_{13}H_8NaO_5Sb$: Sb, 31.3. Found: Sb, 31.5.

(1) (a) A portion of this paper was presented before the XI Annual Convention of the Venezuelan Association for the Advancement of Science, Caracas, April, 1961. (b) From theses submitted by L. C. and J. M. in partial fulfillment of the requirements for the degree of Licenciado de Química, Universidad Central de Venezuela, June, 1961.

(2) H. Causse, *Bull. soc. chim. France*, 245 (1892).

(3) H. P. Brown and J. Austin, *J. Am. Chem. Soc.*, **63**, 2054 (1941).

Methyl Analogs of Papaverine[1a]

JAMES G. BEASLEY AND ALFRED BURGER

Department of Chemistry, University of Virginia, Charlottesville, Virginia

January 15, 1964

Papaverine and papaveraldine analogs which do not contain ether groups in positions 6 and 7 have not been studied widely. Analogs containing methyl instead of methoxyl groups could contribute to such questions as to the significance of methoxy *vs.* methyl groups,[2] or whether the intramolecular distance between the ether oxygens and the isoquinoline nitrogen[3] has a bearing on the pharmacological activity. Several analogs with methyl groups are described.

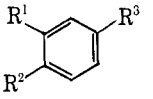
(1) Supported by a grant, NB-01445, from the Institute for Neurological Diseases and Blindness, National Institutes of Health, U. S. Public Health Service.

(2) H. L. Friedman, *Symp. Chem.-Biol. Correlation, Natl. Acad. Sci.-Natl. Research Council*, Washington, D. C., 1951, Publ. No. 206, p. 295.

(3) C. C. Pfeiffer, *Science*, **107**, 94 (1948).

TABLE I

INTERMEDIATES IN SYNTHESES OF PAPAVERINE ANALOGS

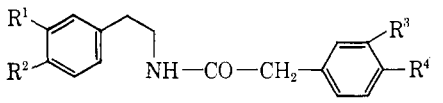


No.	R¹	R²	R³	Yield,[a] %	Recrystn. solvent[b]	M.p., °C.[c]	Empirical formula	—Calcd., %— C	H	—Found, %— C	H
1	OCH_3	CH_3	CHO	51	B[d]	109–110[d]	$C_{15}H_{16}N_2O$[d]	74.97	6.71	74.70	6.68
2	OCH_3	CH_3	CH_2CSN⟨O⟩	76	B	68.5–69.5	$C_{14}H_{19}NO_2S$	63.36	7.21	63.66	7.12
3	OCH_3	CH_3	CH_2CONH_2	64	A	157–158	$C_{10}H_{13}NO_2$	67.02	7.31	66.87	7.43
4	OCH_3	CH_3	$(CH_2)_2NH_3^+Cl^-$	62	C–D	dec.[e]	$C_{10}H_{16}ClNO$[f]	57.00	8.13	57.12	8.06
5	CH_3	CH_3	$(CH_2)_2NH_3^+Cl^-$	69,[g] 65[h]	C–D	dec.[e]	$C_{10}H_{16}ClN$	64.67	8.69	64.42	8.46

[a] Yields are reported for crude products. [b] A, 95% ethanol; B, dilute ethanol; C, absolute ethanol; D, ether. [c] Melting points are for analytical samples. [d] For the phenylhydrazone derivative. [e] No definite melting point observed. [f] Calculated for 0.5 mole of water. [g] Prepared from 3,4-dimethylphenylacetamide. [h] Prepared from 3,4-dimethylphenylacetonitrile.

TABLE II

ANALOGS OF N-(3,4-DIMETHOXYPHENETHYL)HOMOVERATRAMIDE



No.	R¹	R²	R³	R⁴	Method	Yield,[a] %	Recrystn. solvent[b]	M.p., °C.[c]	Empirical formula	—Calcd., %— C	H	—Found, %— C	H
6	CH_3	OCH_3	OCH_3	OCH_3	A, B	21,94	A–B	115–116	$C_{20}H_{25}NO_4$	69.95	7.34	70.02	7.49
7	OCH_3	CH_3	CH_3	CH_3	B	72	C	101.5–102.0	$C_{20}H_{25}NO_2$	77.13	8.09	77.23[d]	8.09[d]
8	CH_3	CH_3	CH_3	CH_3	B	59	A–B	112.5–113.0	$C_{20}H_{25}NO$	81.31	8.53	80.87	8.26
9	CH_3	CH_3	OCH_3	OCH_3	A	77	A–B	115.0–115.5	$C_{20}H_{25}NO_3$	73.36	7.70	72.80	8.21
10	CH_3	OCH_3	CH_3	CH_3	B	38	A–B	111.0–111.5	$C_{20}H_{25}NO_2$	77.13	8.09	76.84	7.99
11	CH_3	OCH_3	CH_3	OCH_3	A	...	A–B	95–96[e]	$C_{20}H_{25}NO_3$	73.36	7.70	73.48	7.83

[a] Yields reported are for crude products. [b] A, benzene; B, petroleum ether (b.p. 60–90°); C, methylcyclohexane. [c] Melting points are for analytical samples. [d] Galbraith Laboratories. [e] M.p. uncorrected.

Experimental[4]

3-Methoxy-4-methylbenzaldehyde.—Reduction of 3-methoxy-4-methylbenzonitrile[5] using Stephen's method[6] yielded 3-methoxy-4-methylbenzaldehyde (1).

3,4-Dimethylacetophenone.—Acetic anhydride (102 g., 1.0 mole) and *o*-xylene (106 g., 1.0 mole) were allowed to react using a general Friedel–Crafts procedure[7] to yield 100 g. (68%) of product,[8] b.p. 94–99° (1.4–2.4 mm.), which was converted directly to the thiomorpholide.[8]

3,4-Disubstituted Phenylacetothiomorpholides.—These compounds, 3,4-dimethylphenylacetothiomorpholide,[8] m.p. 133–137°, and 3-methoxy-4-methylphenylacetothiomorpholide (**2**), were prepared from 3,4-dimethylacetophenone[8] and 3-methoxy-4-methylacetophenone[9] using a Wilgerodt reaction[10] with flowers of sulfur and morpholine.

3,4-Disubstituted Phenylacetic Acids.—Hydrolysis of the 3,4-disubstituted phenylacetothiomorpholides with concentrated sulfuric acid, glacial acetic acid, and water yielded 3,4-dimethylphenylacetic acid,[8,11] m.p. 97–98°, and 3-methoxy-4-methylphenylacetic acid,[12] m.p. 82.5–83.5°.

3,4-Disubstituted Phenylacetamides.—Two amides, 3,4-dimethylphenylacetamide,[11] m.p. 175.5–176.5°, and 3-methoxy-4-methylphenylacetamide (**3**), were synthesized from the corresponding acids by converting them first to acid chlorides with thionyl chloride in benzene and reacting these products with concentrated ammonium hydroxide at 0°.

3,4-Dimethylphenylacetonitrile.—3,4-Dimethylphenylacetamide[11] was dehydrated with phosphorus oxychloride in benzene according to the directions of Delaby, *et al.*[13] The colorless oil boiled at 94–96° (1.1 mm.).

3,4-Disubstituted Phenethylamines.—Three phenethylamine derivatives were prepared. 3-Methoxy-4-methylphenethylamine (**4**) resulted from lithium aluminum hydride reduction[14] of 3-methoxy-4-methylphenylacetamide (**3**). 4-Methoxy-3-methylphenethylamine[15] was obtained as the hydrobromide by reducing 4-methoxy-3-methylphenylacetonitrile[16] with lithium aluminum hydride.[17] The product decomposed with no definite melting point.[15b]

Anal. Calcd. for $C_{10}H_{16}BrNO$: C, 48.79; H, 6.55. Found: C, 48.89; H, 6.68.

3,4-Dimethylphenethylamine (**5**) was synthesized from both 3,4-dimethylphenylacetamide and 3,4-dimethylphenylacetonitrile by the methods cited above

N-(4-Methoxy-3-methylphenethyl)homoveratramide. A.—A solution of 5.0 g. (0.03 mole) of 4-methoxy-3-methylphenethylamine, 7.0 g. (0.03 mole) of crude homoveratroyl chloride [prepared from homoveratric acid (Eli Lilly and Co.) and thionyl chloride in benzene], and 100 ml. of dry benzene was refluxed for 2 hr. Petroleum ether (b.p. 60–90°) was added to the chilled reaction mixture until the mixture became cloudy. Filtration of the thoroughly chilled mixture yielded a tan solid which was

(4) Microanalyses by Barbara Williamson, Judy Jensen, Margaret Logan, and Barbara Zirngibl, except those which are by Galbraith Laboratories, Knoxville, Tennessee; melting points were determined in a standard capillary tube melting point bath and are corrected for stem exposure unless otherwise noted. For details of briefly reported methods see: James G. Beasley, Dissertation, University of Virginia, 1962.

(5) (a) W. Borsche and E. Böcker, *Ber.*, **36**, 4357 (1903); (b) F. Arndt in "Organic Syntheses," Coll. Vol. II, A. H. Blatt, Ed., John Wiley and Sons, Inc., New York, N. Y., 1943, p. 165.

(6) (a) E. Mossetig, *Org. Reactions*, **3**, 246 (1954); (b) R. Gray and J. Bonner, *J. Am. Chem. Soc.*, **70**, 1249 (1948); (c) H. Stephen, *J. Chem. Soc.*, **127**, 1874 (1925).

(7) (a) C. R. Noller and R. Adams, *J. Am. Chem. Soc.*, **46**, 1889 (1924); (b) J. F. Norris and P. Arthur, Jr., *ibid.*, **62**, 874 (1940).

(8) T. Leigh, British Patent 869,504 (1961); *Chem. Abstr.*, **55**, 24796 (1961).

(9) L. Ruzicka and L. Sternback, *Helv. Chim. Acta*, **23**, 355 (1940).

(10) M. Carmack and M. A. Spielman, *Org. Reactions*, **3**, 83 (1946).

(11) G. S. Skinner, T. F. Sanderson, E. R. Bieber, and H. Ewadb, *J. Org. Chem.*, **24**, 403 (1959).

(12) F. Langer and F. Wessely, *Monatsh.*, **88**, 298 (1957); *Chem. Abstr.*, **2**, 312 (1958).

(13) R. Delaby, G. Tsatsas, and Z. Lusinchi, *Compt. Rend.*, **242**, 2644 (1956).

(14) F. Benington, R. D. Morin, L. C. Clark, Jr., and R. P. Fox, *J. Org. Chem.*, **23**, 1979 (1958).

(15) (a) S. N. Sawhney and C. N. Kachru, *J. Indian Chem. Soc.*, **36**, 486 (1959); *Chem. Abstr.*, **54**, 10922 (1960); (b) HCl salt m.p. reported as 240°.

(16) W. Wenner, *J. Org. Chem.*, **16**, 457 (1951).

(17) L. H. Amundsen and L. S. Nelson, *J. Am. Chem. Soc.*, **73**, 242 (1951).

TABLE III
PAPAVERINE ANALOGS

No.	R^1	R^2	R^3	R^4	Y	Salt	Method	Yield,[a] %	Recrystn. solvent[b]	M.p., °C.[c]	Empirical formula	% C Calcd. Found	% H Calcd. Found
12	OCH_3	CH_3	CH_3	CH_3	CH_2	HCl	A, B	39,[d] 98[e]	B	199–200[f,g]	$C_{26}H_{24}ClNO^f$	69.05 69.52	7.53 7.29
13	OCH_3	CH_3	CH_3	CH_3	CO	Picrate	B	...[h]	B	193.5 dec.	$C_{26}H_{24}N_4O_8$	58.21 58.37	4.51 4.51
14	CH_3	CH_3	CH_3	CH_3	CH_2	Picrate	C	<10%	A	207–208 dec.	$C_{26}H_{26}N_4O_7$	61.65 61.29	5.17 5.05
15	CH_3	CH_3	CH_3	CH_3	CO	HCl	C[i]	...	B–C	154–156[j]	$C_{26}H_{22}ClNO^j$	69.45 69.00	6.99 6.93
16	CH_3	OCH_3	OCH_3	OCH_3	CH_2	Picrate	B	63	A	171.5–172.5	$C_{26}H_{26}N_4O_{10}$	56.31 56.10	4.73 4.99
17	CH_3	OCH_3	OCH_3	OCH_3	CO	...	B[k]	...	D	153.0–153.5	$C_{29}H_{29}NO_4$	70.78 71.13	6.24 6.40
18	OCH_3	CH_3	CH_3	CH_3	CO	...	A	59	A	135.5–134.0[l]	$C_{29}H_{19}NO_2$[l]	78.66 78.27	6.27 6.51
19	CH_3	CH_3	CH_3	CH_3	CO	Picrate	A	19	A	186–188	$C_{26}H_{22}N_4O_8$	60.23 60.51	4.28 4.59
20	CH_3	OCH_3	OCH_3	OCH_3	CH_2	Picrate	B	41[d]	A	193.5–194.0	$C_{26}H_{24}N_4O_{10}$	56.52 56.47	4.38 3.98
21	CH_3	OCH_3	OCH_3	OCH_3	CO	...	B	..	A	177.0–178.5	$C_{29}H_{19}NO_4$	71.20 70.65	5.68 5.73

[a] Yields are reported for crude products. [b] A, 95% ethanol; B, dilute ethanol; C, ether; D, methylcyclohexane. [c] Melting points are for analytical samples. [d] Yields of free base. [e] Calculated for 1 mole of H_2O. [f] M.p. of monohydrate; anhydrous salt melted at 197.5–198.5°. [g] Picrate melted at 181–182°. *Anal.* Calcd. for $C_{26}H_{26}N_4O_8$: C, 59.76; H, 5.02. Found: C, 59.21; H, 4.95. [h] Obtained as a by-product when compound **12** was neutralized for dehydrogenation. [i] Obtained by chromatographic elution of compound **14** (crude free base) in $CHCl_3$ on an alumina column. [j] M.p. of monohydrate. [k] Obtained by chromatographic elution of compound **16** (crude free base) in $CHCl_3$ on an alumina column. [l] This compound was also analyzed as the oxime, m.p. 305–310°. *Anal.* Calcd. for $C_{29}H_{20}N_2O_2$: C, 74.97; H, 6.29. Found: C, 74.74; H, 6.21.

washed successively with 30-ml. portions of 5% hydrochloric acid, 5% sodium carbonate solution, and water. The product (**6**) (2.1 g., 21%), m.p. 115–116°, was recrystallized from benzene–petroleum ether.

B.—Dobrowsky's method[18] for producing amides by refluxing free amine with equimolar quantities of organic acid in dry decalin, gave a 94% yield of crude product and an 85% yield of purified product.

Substituted 3,4-Dihydroisoquinolines.—Substituted 3,4-dihydroisoquinolines (**12, 14,** and **16**) were synthesized using essentially the Bischler-Napieralski reaction[19] with varying solvents, reaction times, and cyclizing agents.

As noted by several authors,[20] air oxidation of 1-benzyl-3,4-dihydroisoquinolines (**12, 14,** and **16**) to 1-benzoyl-3,4-dihydroisoquinolines (**13, 15,** and **17**) took place with ease in neutral or alkaline media. Similar oxidations occurred also in some cases, in the aromatic 1-benzylisoquinoline series (*e.g.*, **20**), as had been observed in other instances by Buck, *et al.*[20a]

A.—To a suspension of 0.5 g. of the substituted phenethylacetamide in 3 ml. of dry xylene,[21] 4 ml. of freshly distilled phosphorus oxychloride was added. The resulting mixture was refluxed for 3.5 hr. and cooled to room temperature. Cold water (200 ml.) was added and the mixture was stirred until hydrolysis was complete. The chilled mixture was extracted with ether to remove unchanged amide and then neutralized cautiously with 40% sodium hydroxide solution. Extraction of the alkaline solution with ether was followed by drying over solid potassium hydroxide pellets. Evaporation of this dried extract yielded the crude product, usually as a viscous oil. The oil was converted to an appropriate salt.

B.—This method was adapted from that of Frydman, *et al.*,[22] and employed 0.5 g. of amide, 5 ml. of chloroform, and 1 g. of phosphorus pentachloride. When an alkoxyl group was present in a position *para* to the site of ring closure, a reaction temperature of 0° for 3 hr. then 26° for 4 days was used. When the *para* substituent was an alkyl group, the mixture was refluxed for 14 hr. The solvent was then removed by evaporation and the residue treated as in method A.

C.—In this procedure 2.5 g. of amide was heated with 15.8 g. of polyphosphoric acid[23] and 2.5 g. of phosphorus oxychloride for 14 hr. on a water bath. The resulting reaction mixture was then worked up as described in method A.

Dehydrogenation of Substituted 3,4-Dihydroisoquinolines. A.—To a solution containing 1.0 g. of crude substituted 3,4-

(18) A. Dobrowsky, *Monatsh.*, **82**, 122 (1951).

(19) (a) W. M. Whaley and T. R. Govindachari, *Org. Reactions*, **6**, 75 (1951); (b) A. Bischler and B. Napieralski, *Ber.*, **26**, 1903 (1893).

(20) (a) J. S. Buck, R. D. Haworth, and W. H. Perkin, Jr., *J. Chem. Soc.*, **125**, 2176 (1924); (b) A. Lindemann, *Helv. Chim. Acta*, **32**, 60 (1949); (c) R. S. Livshits, G. I. Bazilevskaya, M. S. Baimova, O. E. Dobrovinskaya, and N. A. Prenbrazhenskii, *Zh. Obshch. Khim.*, **17**, 1671 (1947); *Chem. Abstr.*, **42**, 2606 (1948).

(21) S. Sugasawa and H. Yoshikawa, *J. Chem. Soc.*, 1583 (1933).

(22) B. Frydman, R. Bendish, and V. Deulofeu, *Tetrahedron*, **4**, 342 (1958).

(23) (a) C. R. Hauser and J. G. Murray, *J. Am. Chem. Soc.*, **77**, 2854 (1955); (b) H. Singer and W. Shive, *J. Org. Chem.*, **22**, 84 (1957); (c) F. Uhlig, *Angew. Chem.*, **66**, 435 (1954); (d) We are indebted to the Victor Chemical Works, Chicago, Ill., for a generous sample of polyphosphoric acid.

dihydroisoquinoline in 40 ml. of dry cymene was added 4.0 g. of 5% palladium on deaerated charcoal.[24] The reaction mixture was then refluxed vigorously for 4 hr., filtered while hot, and the residue was washed thoroughly with chloroform. After combining the filtrates, 300 ml. of ether was added and the solution was extracted twice with 50-ml. portions of 20% hydrochloric acid.[25] The chilled acidic solution was neutralized cautiously with 40% sodium hydroxide solution and the product was extracted with two 100-ml. portions of ether. The ethereal extract was dried over potassium hydroxide and evaporated to yield the substituted isoquinoline. This material was then recrystallized or converted to a salt.

B.—A mixture of 0.5 g. of the crude substituted 3,4-dihydroisoquinoline and 0.5 g. of 5% palladium on charcoal[24] was placed under vacuum (0.1–0.3 mm.) and heated slowly to 150°. A temperature of 150–210° was maintained for 3 hr. The reaction mixture was cooled to 25° and extracted with ethanol. Concentration of the ethanolic solution yielded the crude substituted isoquinoline.

(24) R. Mozingo in "Organic Syntheses," Coll. Vol. III, E. C. Horning, Ed., John Wiley and Sons, Inc., New York, N. Y., 1955, p. 685.

(25) The substituted isoquinoline hydrochlorides were found to be very soluble in benzene-type solvents and it was necessary to dilute with ether before effective extraction with hydrochloric acid could be obtained.

Isolation of Cephalosporin C

E. L. PATTERSON, J. C. VAN METER, AND N. BOHONOS

Biochemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

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We wish to report a convenient procedure for the isolation of cephalosporin C,[1] which in turn may serve as a source of 7-aminocephalosporanic acid for the exploration of potentially useful semisynthetic cephalosporins.

Experimental

Cephalosporin C was measured by a cup agar diffusion assay *vs. Salmonella gallinarum* grown on pH 6 nutrient agar[2] medium containing penicillinase[3] (1250 μ/ml.) to destroy penicillin N.

A selected strain of *Cephalosporium acremonium* CMI 49,137 was grown at 28° in a 300-l. stainless steel fermentor with agitation and aeration on a medium composed of corn starch, 3%; soybean meal, 1%; calcium carbonate, 0.75%; DL-methionine, 0.1%; potassium chloride, 0.05%; and magnesium sulfate, 0.025%.

Following 3 days of fermentation, the culture mash was filtered. Activated carbon (3%) was added to the filtrate (275 l.) which was brought to pH 2.5 with hydrochloric acid. After stirring for 30 min., the carbon was filtered with the aid of diatomaceous earth and washed with water. The filter cake was stirred for 30 min. in 100 l. of acetone–water (3:2) maintained at pH 4.5 with ammonium hydroxide. The solids were removed by filtration and the eluate was concentrated to 60 l. under reduced pressure. The concentrate was adjusted to pH 2.5 with Dowex-50 (H^+) and stored at 4° overnight to destroy penicillin N, coproduced in the fermentation. After raising the pH to 4.5 with ammonium hydroxide, the solution was percolated through 6 kg. of moist IRA-401 ($HCOO^-$) (20 to 50 mesh) in a 15.3-cm. (i.d.) column (flow rate 20 l./hr.). The resin column was rinsed with water and developed with 30 l. of 0.2 *M* ammonium formate (flow rate 8 l./hr.). The eluate fractions containing cephalosporin C were combined and diluted with 1.5 vol. of acetone.

Aluminum oxide (2 kg., chromatographic grade, Merck) was equilibrated with aqueous formic acid pH 6, washed with acetone, and air-dried. This was packed in a 7-cm. (i.d.) column. The aqueous acetone solution was applied to the column (flow rate 12 l./hr.) followed by an acetone–water (3:2) wash. The column was developed with 0.05 *M* ammonium formate (flow rate 7 l./hr.) and the portion of the eluate containing cephalosporin C was lyophilized. The residue in water (250 mg./ml.) was chilled and 2 vol. of ethanol was slowly added. Cephalosporin C ammonium salt crystallized at 4° overnight and was recrystallized from water–ethanol; $[\alpha]^{25}D$ +104° (*c* 2.2, water); λ_{max} 260 mμ (ϵ_{max} 9300, water).

Anal. Calcd. for $C_{16}H_{24}N_4O_8S \cdot H_2O$: C, 42.66; H, 5.77; N, 12.45; S, 7.11. Found: C, 42.82; H, 5.96; N, 12.11; S, 7.01.

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(1) (a) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **62**, 651 (1956); (b) E. P. Abraham and G. G. F. Newton, *ibid.*, **79**, 377 (1961).

(2) No. 0634, Difco Laboratories, Detroit, Mich.

(3) Baltimore Biological Laboratory, Baltimore, Md.

Book Reviews

Vitamins and Coenzymes. By ARTHUR F. WAGNER and KARL FOLKERS, Merck Sharp and Dohme Research Laboratories. Interscience Publishers (John Wiley and Sons, Inc.), New York, N. Y. 1964. xvi + 532 pp. 23.5 × 16 cm. $17.50.

Although several annual progress-series are available in the field of vitamins and similar biocatalysts, few books have attempted to cover all of the vitamins and their activated forms in a compact volume. The last of these books (by Rosenberger) had been written over 10 years ago, and some of the more complex observations in this field have been made since then. Another large monograph has dealt with the nutritional aspects of the vitamins only. In 1960, Folkers and Wagner contributed a massive chapter on vitamins to "Medicinal Chemistry" (A. Burger, Ed., Interscience Publishers), and this chapter has now been expanded to the present volume.

The authors are organic chemists with a deep understanding of biochemistry, and this background colors their presentation. Methods of isolation, structural proof, and synthesis are reviewed broadly, leading from historical introductions up to the latest chemical data. However, biochemical theories and relations to coenzymes form a solid background to the chemical work, and nutritional and clinical applications are summarized clearly, concisely, and in depth. The complicated stereochemistry of some of the vitamins is presented masterfully as may be expected from an author such as Karl Folkers who has been a leading figure in this field for decades.

This book promises to become the "bible" on vitamins and their biochemical derivatives, and to remain so for years to come. Its publication at this moment is particularly timely because many—though by no means all—major problems of vitamin chemistry have been worked over extensively, and there seems to be a temporary hiatus in the dynamic progress of this field. By summarizing critically what has been done, and pointing out what needs to be done, the authors may well catalyze a revival of intensive activity in the remaining structural and biochemical problems of the vitamin field and in the largely untouched area of selective vitamin antagonists.

UNIVERSITY OF VIRGINIA ALFRED BURGER
CHAROLOTTESVILLE, VIRGINIA