was treated with lithium aluminum hydride in the presence of aluminum chloride to maintain acidity. During the decomposition of the hydride complex the pH of the solution was never allowed to rise above 5. The product, isolated by continuous extraction with ethyl acetate, was 87 mg. of 6-methylthiopurine.

A control experiment with all reagents except the lithium aluminum hydride showed that VII was not hydrolyzed under the acid conditions maintained. Therefore, since hydrolysis is not the cause of the formation of 6-methylthiopurine, hydrogenation must have occurred.

1,4-Bismethanesulfonates of the Stereoisomeric Butanetetraols and Related Compounds

PETER W. FEIT

Leo Pharmaceutical Products, Ballerup, Denmark

The synthesis of the stereoisomeric butane-1,2,3,4-tetraol 1,4-bismethanesulfonates and some of their cyclic acetals and ketals is described. The preparation of L-threitol 1,4-bismethanesulfonate from L(+)-tartaric acid as starting material makes this compound easily available for clinical evaluation.

The synthesis and cytoactivity of D-mannitol 1,6-bismethanesulfonate has led to a more detailed investigation of methanesulfonated sugars and polyhydric alcohols which are considered water-soluble analogs of 1,4-dimethanesulfonyloxybutane (busulfan).[1-3]

As already briefly reported we[4] synthesized the 1,4-bismethanesulfonates of the stereoisomeric 1,2,3,4-butanetetraols (I).

$$CH_3SO_2OCH_2CHOHCHOHCH_2OSO_2CH_3 \qquad I$$
$$L, (D, DL, meso)$$
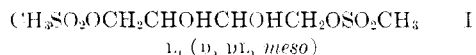
We suggested that these compounds, although identical with busulfan with respect to their chain length, might show an alkylating mechanism different from that of busulfan owing to the hydroxyl groups in the α-position to the sulfonyloxy groups. Furthermore, our interest in these compounds was based on the known correlation in the busulfan series $(CH_3SO_2O(CH_2)_{2-10}OSO_2CH_3)$ between chain length and both water–ether solubility ratio and the biological activity.[5] In addition, it could be expected that the stereoisomers would be different in their alkylation reactions *in vivo*.

In continuation of our search we prepared certain cyclic acetals and ketals (1,3-dioxolanes) of I with the purpose of obtaining substances for investigation as anticancer agents. Such dioxolanes may be regarded either as camouflaged 1,4-bismethanesulfonates of butanetetraol with initially decreased water solubility, or alternatively, when no ring hydrolysis is assumed, as bismethanesulfonates with a fixed distance between the alkylating groups. The latter assumption means that only the compounds with an *erythro-*, but not with a *threo-*configuration, have the ability to react under

cycloalkylation, and this is proved to be the main reaction of busulfan *in vivo*.[6]

Of course, both the solubility of these compounds and the stability of the ring will depend on the nature of the substituents originating from the aldehyde or ketone forming the basis for the dioxolane. Moreover, the ring formation should influence the alkylating properties of these bismethanesulfonates.

Chemistry.—The synthetic routes for the preparation of the stereoisomeric bismethanesulfonates I are summarized for the L-isomers. Since no attack on the asymmetric carbon atoms is involved, the configuration of the compound is given by that of the starting material. The ring opening of the stereoisomeric 1,2:3,4-diepoxybutanes (VII) with methanesulfonic acid resulted in yields of *ca.* 30% of the corresponding I when the reaction was carried out in a diethyl ether-*t*-butyl alcohol mixture. Using pure diethyl ether as the solvent only small quantities of *meso*-I were obtainable, which is in agreement with recently reported results.[3]

The reaction of the stereoisomeric 1,4-dibromo-2,3-butanediols (VI) with silver methanesulfonate in boiling acetonitrile gave the corresponding I in 30–40% yield. To develop the preparation of L-I on a larger scale and consequently to make a clinical evaluation of the compound possible, the synthesis was based on L(+)-tartaric acid as an easily available starting material. Diethyl L-tartrate (II), obtained from natural tartaric acid by azeotropic esterification, was converted into diethyl 2,3-O-isopropylidene-L-tartrate (III) by an acid-catalyzed reaction with acetone in low boiling petroleum ether under simultaneous azeotropic removal of the reaction water. This procedure proved superior to the previously described ketalization with copper sulfate as a water-removing agent.[7] Compound III was then reduced with LiAlH4 to 2,3-O-isopropylidene-L-threitol (IV).[8] Ketal hydrolysis was avoided by an alkaline isolation

(1) A. Haddow, G. M. Timmis, and S. S. Brown, *Nature*, **108**, 1164 (1958); L. Vargha and J. Kuszmann, *Naturwissenschaften*, **46**, 84 (1959); B. Kellner and L. Németh, *Brit. J. Cancer*, **13**, 469 (1959); L. Vargha, L. Toldy, G. Fehér, T. Horváth, E. Kasztreiner, J. Kuszmann, and S. Lendvai, *Acta Physiol. Acad. Sci. Hung.*, **19**, 305 (1961).

(2) L. Vargha, O. Fehér, T. Horváth, L. Toldy, and J. Kuszmann, *Acta Chim. Hung.*, **25**, 361 (1960).

(3) S. S. Brown and G. M. Timmis, *J. Chem. Soc.*, 3656 (1961).

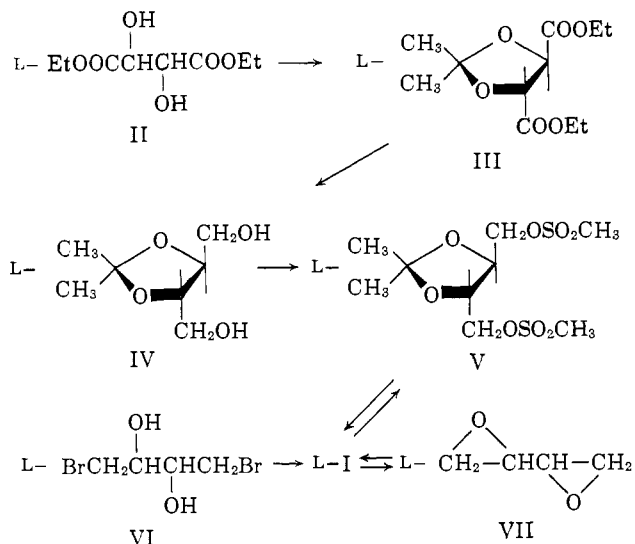(4) P. W. Feit, *Tetrahedron Letters*, 716 (1961).

(5) R. F. Hudson, G. M. Timmis, and R. D. Marshall, *Biochem. Pharmacol.*, **1**, 48 (1958).

(6) J. J. Roberts and G. P. Warwick, *Biochem. Pharmacol.*, **6**, 217 (1961).

(7) Y. Tsuzuki, *Bull. Chem. Soc. Japan*, **10**, 255 (1935); H. J. Lucas and W. Baumgarten, *J. Am. Chem. Soc.*, **63**, 1653 (1941).

(8) The preparation of IV from 3,4-O-isopropylidene-D-mannitol in several steps has been described.[9]

(9) L. J. Rubin, H. A. Lardy, and H. O. L. Fischer, *J. Am. Chem. Soc.*, **74**, 425 (1952).

OH
|
L- EtOOCCHCHCOOEt → L-
|
OH

II

III

IV → V

L- BrCH₂CHCHCH₂Br → L-I ⇄ L- CH₂—CHCH—CH₂

VI   VII

The compound had inhibitory effects on several rat tumors and produced cures of both recently implanted and established Lymphoma 8. After 210 mg./kg./day i.p. or 235 mg./kg./day p.o. for 5 days started the day after transplantation of Dunning Leukemia, 90% of the treated animals were without tumors at day 31. The LD_{10}/ED_{90} is 2 for the latter system. The compound is now being subjected to clinical evaluation.

process. The reaction of IV with methanesulfonyl chloride in pyridine yielded 2,3-O-isopropylidene-L-threitol 1,4-bismethanesulfonate (V) which proved identical with material produced from L-threitol via a mono-O-isopropylidene-L-threitol mixture.[10] As an acid-catalyzed hydrolysis of 2-dialkyl substituted dioxolanes proceeds easily and under conditions which leave primary methanesulfonyloxy groups intact, L-threitol 1,4-bismethanesulfonate (L-I) was obtainable in excellent yields from V. By this reaction route the conversion of racemic and meso-tartaric acid to the corresponding I could also be effected, and the acetone was also replaceable by other ketones or aldehydes.

It is noteworthy that under our reaction conditions, after prolonged reaction time, diethyl meso-tartrate was capable of forming a 2,3-O-isopropylidene compound (erythro-hydroxy pair) which after the LiAlH₄-reduction makes the 2,3-O-isopropylideneerythritol available.

Treatment of L-I in ethereal solution with concentrated potassium hydroxide results in L-1,2:3,4-diepoxybutane (VII). This sequence makes the preparation of VII possible without resolution.[11]

The last synthetic route to I described previously consequently made possible the isolation of various cyclic acetals and ketals of I as intermediates. However, we found it convenient to prepare these dioxolanes by acetalization and ketalization reactions using the corresponding I as starting material as it was abundantly at our disposal via its 2,3-O-isopropylidene derivative. Either the free carbonyl compound or its methyl or ethyl acetals were employed. By selecting suitable reaction conditions this reaction was easily carried out with the meso compound (erythro-hydroxy pair). The cyclic acetals and ketals thus obtained are listed in Table II.[12]

Anticancer Screening.—All the bismethanesulfonates described in Tables I and II were submitted to anticancer screening at the Cancer Chemotherapy National Service Center, U. S. Public Health Service. The antitumor activity and pharmacology of L-threitol 1,4-bismethanesulfonate (L-I) have recently been reported.[13]

Experimental[14]

Diethyl 2,3-O-Isopropylidene-L-tartrate.—A mixture of diethyl-L-tartrate[15] (157 g.), acetone (240 ml.), petroleum ether (b.p. 40–60°, 410 ml.), and sulfuric acid (0.25 ml.) was refluxed under a continuous water separator for 9 days. The solvents were removed and the residue worked up as described earlier[5] yielding 155 g. of analytically pure compound.

2,3-O-Isopropylidene-L-threitol.—A suspension of LiAlH₄ (42 g.)[16] in diethyl ether (400 ml.) was refluxed for 30 min. with vigorous stirring. A solution of diethyl 2,3,-O-isopropylidene-L-tartrate (123 g.) in diethyl ether (500 ml.) was added dropwise without heating over a period of about 2 hr., the heat of reaction causing a gentle refluxing. After additional heating for 3 hr. ethyl acetate (50 ml.) was carefully added, and the reaction mixture cooled to 0–5°. After successive cautious additions of water (42 ml.), 4 N NaOH (42 ml.), and water (130 ml.), the inorganic precipitate which had formed was removed by filtration, and extracted thoroughly with ether (Soxhlet). The combined ethereal extracts were dried (MgSO₄) and evaporated under reduced pressure. Distillation of the residue yielded 2,3-O-isopropylidene-L-threitol (64 g.), b.p. 96–96.5° (0.5 mm.) [lit.[9] b.p. 91–93° (0.01–0.02 mm.).

In parallel charges the compound crystallized. After recrystallization from diisopropyl ether, it melted at 49.5–51°; $[\alpha]^{20}D$ +4.1° (c 5, chloroform); for D-isomer lit.[9] m.p. 48–51°; $[\alpha]^{20}D$ −3.1° (c 5.2, chloroform).

Diethyl 2,3-O-Isopropylidene-meso-tartrate.—Crude crystalline diethyl meso-tartrate obtained by azeotropic esterification of meso-tartaric acid monohydrate (100 g.) was reacted with acetone as described for the corresponding L-compound. It was necessary to extend the reaction time to 12 days. The product (100 g.) had b.p. 136–140° (10 mm.).

Anal. Calcd. for $C_{11}H_{18}O_6$: C, 53.65; H, 7.37. Found: C, 53.85; H, 7.43.

2,3-O-Isopropylideneerythritol.—Diethyl 2,3-O-isopropylidene-meso-tartrate (61.59 g.) was reduced with LiAlH₄ (12.5 g.) as described for the L-compound to yield 2,3-O-isopropylideneerythritol (26.6 g.); b.p. 106–108° (0.4–0.5 mm.); m.p. 40–46°. After several recrystallizations from diisopropyl ether the m.p. was raised to 48–49.5°.

(10) P. W. Feit, Chem. Ber., 96, 712 (1963).

(11) P. W. Feit, ibid., 93, 116 (1960).

(12) J. Tsuzuki, Bull. Chem. Soc. Japan, 11, 362 (1936).

(13) R. Jones, W. B. Kessler, H. E. Lessner, and L. Rane, Cancer Chemotherapy Rept., 10, 99 (1960); F. R. White, ibid., 24, 95 (1962).

(14) Analyses by Mr. G. Cornali and W. Egger of these laboratories. Melting points were taken in open glass capillaries and rounded off to half degrees, using a Hershberg apparatus with thermometers subdivided in 0.1°. Technical assistance: Th. Rolle and W. Schlichtkrull.

(15) Prepared in 89% yield by azeotropic esterification with petroleum ether (b.p. 40–60°) as azeotropic component.
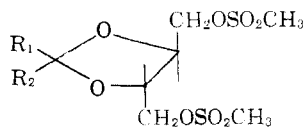
(16) The amount of LiAlH₄ amount could be reduced to 24 g. without loss in yield.

Table II

Cyclic Acetals and Ketals of d-, l-, dl- Threitol- and Erythritol 1,4-Bismethanesulfonate

Config-uration of the basic sugar-alcohol	R₁	R₂	M.p., °C	Solvent of recrystn.	[α]²⁰D (c 2, acetone)	Method[a]	Formula	% Calcd. C	H	S	Halo-gen	% Found C	H	S	Halo-gen
dl	H	H	89.0-90.5	Chloroform	---	A	C₇H₁₄O₈S₂	28.96	4.89	22.00		29.11	4.54	22.02	
d	H	H	93.5-95.5	Chloroform	+44.9	B						28.99	4.89	22.01	
l	H	H	94.5-95.5	Chloroform	-45.2	A, B						28.86	4.89	22.00	
meso	H	H	110.5-112.0	Chloroform	---	B						28.61	4.84	21.98	
d	CH₃	H	109.0-110.5	Chloroform	+39.0	B	C₈H₁₆O₈S₂	31.57	5.30	21.07		31.63	5.40	21.12	
l	CH₃	H	108.5-110.5	Chloroform	-37.1	B						31.49	5.34	21.15	
meso	CH₃	H	108.5-110.0	Chloroform	---	B						31.36	5.50	21.27	
Isomers	CH₃	CH₃	See Experimental												
d	CCl₃	H	105.5-106.5	Chloroform (warm)-ether	+22.5	A	C₈H₁₃Cl₃O₈S₂	23.57	3.21	15.72	26.09	23.64	3.24	15.80	26.34
l	CCl₃	H	105.0-106.5	Chloroform (warm)-ether	-21.8	A						23.54	3.27	16.08	26.19
meso	CCl₃	H	157.5-159.5	Ethyl acetate	---	A						23.61	3.35	15.96	26.24
l	CBr₃	H	110.5-112.5	Chloroform (warm)-ether	-11.0	A	C₈H₁₃Br₃O₈S₂	17.75	2.42	11.85	44.31	18.01	2.58	11.82	44.22
l	CF₃	H	68.0-69.0	Ethanol	-32.2	C	C₈H₁₃F₃O₈S₂	26.81	3.66	17.90		27.02	3.72	17.87	
d	-(CH₂)₅-	--	95.5-96.5	Chloroform-ether	+20.4	D	C₁₂H₂₂O₈S₂	40.21	6.19	17.89		40.01	6.36	17.91	
l	-(CH₂)₅-	--	95.5-97.0	Chloroform-ether	-20.4	D						40.37	6.30	17.76	
meso	-(CH₂)₅-	--	79.0-80.5	Chloroform-ether	---	D						40.7	6.25	17.70	
d	C₆H₅	H	116.0-118.0	Chloroform	+13.8	D	C₁₃H₁₈O₈S₂	42.61	4.35	17.50		42.49	4.87	17.69	
l	C₆H₅	H	116.0-117.5	Chloroform	-14.0	D						42.40	4.86	17.58	
dl	C₆H₅	H	79.0-81.0	Chloroform	---	D						42.69	5.05	17.69	
d	CH₂C₆H₅	H	80.0-81.5	Chloroform	+18.6	E	C₁₄H₂₀O₈S₂	44.20	5.30	16.85		44.21	5.35	16.99	
l	CH₂C₆H₅	H	80.0-81.5	Chloroform	-19.0	E						44.21	5.37	16.97	

[a] The letters relate to the general procedures in the Experimental part. [b] The same physical properties were obtained for this compound prepared from diethyl 2,3-O-methylene tartrate,[12] by the procedure described for the corresponding 2,3-O-isopropylidene compound. [c] (c 1, acetone). [d] By applying the procedure resulting in 2,3-O-isopropylidene-L-threitol 1,4-bismethanesulfonate from diethyl tartrate, the corresponding 2,3-O-phenylethylidene compound was obtained with the same physical properties.

Anal. Calcd. for C₇H₁₄O₆: C, 51.84; H, 8.70. Found: C, 51.48; H, 8.85.

2,3-O-Isopropylidene-L-threitol 1,4-Bismethanesulfonate. (a). —To a solution of 2,3-O-isopropylidene-L-threitol (110 g.) in pyridine (500 ml.), methanesulfonyl chloride (130 ml.) was added dropwise while stirring at −10 to −5° over a period of 1.5 hr. After standing for 20 hr. at about −5° the reaction mixture was poured into ice-water (1.5 l.). The resulting precipitate was washed with water and dried in vacuo. The crude product was recrystallized from chloroform-diethyl ether, yield 177.6 g., [α]²⁰D −21.3 (c 2, acetone). The compound proved to be identical with that isolated after mesylation of the 1,2- and 2,3-O-isopropylidene-L-threitol mixture[10] and could be obtained in two crystal modifications (or mixtures of these) with m.p. 79-80.5° and 85.5-86.5°, respectively, with identical analyses and infrared spectra in CS₂ solution.

(b).—A mixture of L-threitol 1,4-bis-methanesulfonate (27.8 g.), acetone (100 ml.), chloroform (100 ml.), and a few drops of methanesulfonic acid was gently refluxed for 3 hr. During this period a solvent azeotrope was distilled very slowly while at the same rate a mixture of acetone-chloroform (1:1) was added. The resulting clear solution was concentrated to 100 ml., cooled, and after addition of about 100 ml. of diethyl ether yielded 31.2 g. of material. After being recrystallized 3 times from chloroform-diethyl ether the compound had m.p. 85.5-86.5° (see a). The infrared spectrum in CS₂ was identical with that of the compound prepared as in a.

Anal. Calcd. C₉H₁₈O₈S₂: C, 33.95; H, 5.70; S, 20.14. Found: C, 33.71; H, 5.66; S, 20.14.

2,3-O-Isopropylidene-D-threitol 1,4-Bismethanesulfonate.—This compound was prepared analogous with the L-isomer from D-threitol 1,4-bismethanesulfonate with m.p. 85.5-86.5° after

recrystallization from chloroform-diethyl ether: [α]²⁰D +21.9 (c 2, acetone).

Anal. Found: C, 34.04; H, 5.81; S, 20.19.

2,3-O-Isopropylidene-DL-threitol 1,4-Bismethanesulfonate.—This compound was obtained from DL-tartaric acid by the procedure described for the L-isomer and had m.p. 95.5-97.5° after repeated recrystallizations from chloroform-diethyl ether.

Anal. Found: C, 34.07; H, 5.77; S, 20.07.

2,3-O-Isopropylideneerythritol 1,4-Bismethanesulfonate. —This compound was prepared either from erythritol 1,4-bis-methanesulfonate or from 2,3-O-isopropylideneerythritol as described for the corresponding L-threitol compound. After recrystallization from chloroform-diethyl ether the m.p. was 94.5-96.5°.

Anal. Found: C, 33.84; H, 5.71; S, 20.16.

DL-, L- or D-Threitol 1,4-bis-methanesulfonate; Erythritol 1,4-Bismethanesulfonate. (a) From DL-, L-, D-, or meso-1,4-Halogeno-2,3-butanediol.—A mixture of the corresponding 1,4-dibromo-2,3-butanediol[11] (12.4 g.), silver methanesulfonate (30 g.), and acetonitrile (54 ml.) was refluxed for 1 hr. The silver halide thereby formed was removed by filtration and washed with warm acetonitrile. From the combined filtrates the solvent and diacetyl were distilled under reduced pressure, and the residue was then extracted with ice-cold acetone (70 ml.) leaving the excess silver methanesulfonate. The acetone was removed by distillation in vacuo and the crude bismethanesulfonate crystallized when treated with diethyl ether (about 100 ml.). Repeated recrystallizations from acetone-diethyl ether followed by ethanol gave the desired compound in a 30-40% yield. The physical properties were identical with those of the corresponding compounds obtained by one of the other routes and are listed in Table I.

(b) **From DL-, L-, D-, or *meso*-1,2:3,4-Diepoxybutane.**—To a stirred and cooled mixture of *t*-butyl alcohol (100 ml.) and diethyl ether (100 ml.), methanesulfonic acid (technical grade, 90%, 80 ml.) was added. A solution of the corresponding 1,2:3,4-diepoxybutane[11] (32 ml.) in diethyl ether (100 ml.) was then added dropwise over 2.5 hr. at 15–20°. After 33% of the diepoxybutane had been added the bismethanesulfonate began to crystallize from the reaction mixture. After the addition was completed, stirring was continued for 5 hr. followed by standing in a refrigerator for 16 hr. The crude product was collected by filtration, and washed with a 1:2 mixture of *t*-butyl alcohol–diethyl ether. After several recrystallizations from ethanol, the yield of pure compound was approximately 30% based on diepoxybutane. A higher yield was obtained of the *meso* isomer. The physical properties were identical with those of the corresponding compound obtained by one of the other routes and are listed in Table I.

(c) **From 2,3-O-Isopropylidene-DL-, L-, or D-Threitol 1,4-Bismethanesulfonate or 2,3-O-Isopropylideneerythritol 1,4-Bismethanesulfonate.**—The corresponding 2,3-O-isopropylidene compound (100 g.) was refluxed in 96% ethanol (400 ml.) for 10 hr., after addition of methanesulfonic acid (0.5 ml.). The desired bismethanesulfonate crystallized on cooling and was filtered and washed with ethanol and diethyl ether. After recrystallization from ethanol the yield exceeded 90%, and the physical properties were identical with those of the corresponding compound obtained by one of the other methods; see Table I.

L-1,2:3,4-Diepoxybutane.—A suspension of L-threitol 1,4-bismethanesulfonate (55.8 g.) in diethyl ether (150 ml.) was treated with KOH (25 g.) in water (25 ml.) as described[11] for L-2,3-dibromo-1,4-butanediol resulting in L-1,2:3,4-diepoxybutane (12.8 g.) proved to be identical with authentic material.[11]

General Methods for the Preparation of Cyclic Acetals and Ketals of D-, L-, DL-Threitol- and Erythritol 1,4-Bismethanesulfonates. A.—A mixture of the corresponding bismethanesulfonate (10 g.), concentrated sulfuric acid (20 ml.), and the aldehyde (20% excess; formaldehyde as (CH₂O)ₓ with 100–200% excess)

was heated to 60–70° while stirring for 15–30 min. After cooling, the reaction mixture was poured into ice–water, and the reaction product isolated by filtration or extraction with chloroform or ethyl acetate. The product was then dried and the extraction solvent evaporated. For solvents of recrystallization and physical properties see Table II.

B.—The corresponding bismethanesulfonate (10 g.) was refluxed in excess diethyl acetal for 6–20 hr. after methanesulfonic acid had been added (5 drops). If acetals with a higher boiling point were used, then dilution with chloroform or ethyl acetate (100–200 ml.) was necessary. As for the *meso* isomers, an occasional removal of a solvent–ethanol mixture by distillation was advantageous. After cooling, the reaction product was crystallized by addition of diethyl ether. For solvents of recrystallization and physical properties see Table II.

C.—A mixture of L-threitol 1,4-bismethanesulfonate (12 g.), concentrated sulfuric acid (50 ml.), and α-ethoxy-β-trifluoroethanol (7 g.) was stirred at 70° for 1 hr. and the reaction mixture treated as described in A. The resulting oil was crystallized by treatment with diethyl ether. For the solvent of recrystallization and physical properties see Table II.

D.—To a suspension of the corresponding bismethanesulfonate (10 g.) in a solution of the aldehyde or ketone (50–100% excess) in chloroform or benzene (100 ml.) was added a few drops of methanesulfonic acid, and the mixture was refluxed under a continuous water separator for 3–20 hr. After cooling and dilution with diethyl ether the crude product was isolated by filtration (see Table II).

E.—To a suspension of the corresponding bismethanesulfonate (10 g.) in benzene (100 ml.) was added a few drops of methanesulfonic acid, and the mixture refluxed for 2 hr. while a solution of phenylacetaldehyde dimethylacetal in benzene (200 ml.) was added dropwise. During the reaction a methanol–benzene mixture was removed by distillation resulting in a clear solution (60 ml.). After cooling and dilution with diethyl ether the crude product was isolated by filtration (see Table II).

Vitamin B₆ Analogs. II.[1,2] Synthesis of 4,6-Dimethyl-5-mercapto-3-pyridinemethanol and of 5-Mercapto-6-methyl-3,4-pyridinedimethanol Hydrochlorides

JOSEPH L. GREENE, JR., AND JOHN A. MONTGOMERY

*Kettering-Meyer Laboratory (Affiliated with Sloan-Kettering Institute),
Southern Research Institute, Birmingham 5, Alabama*

The 3-mercapto analogs of pyridoxine and 4-desoxypyridoxine have been prepared by the reaction of the appropriate diazonium salt with potassium ethyl xanthate followed by reduction of the xanthate ester with lithium aluminum hydride. These compounds and the requisite intermediates that have been evaluated showed no anti-B₆ activity or antitumor activity. "3-Thiopyridoxine" was capable of replacing B₆ for growth probably by conversion to pyridoxine.

The rationale underlying the synthesis of potential vitamin B₆ antagonists for evaluation as antitumor agents has been discussed.[1] Briefly, the striking inhibition of 4-desoxypyridoxine, alone or in combination with certain other compounds, on the growth of Sarcoma 180 (in Swiss mice maintained on a B₆-deficient diet[3]) encouraged us to search for better B₆ antagonists that might be effective antitumor agents on a complete diet.

One of our early candidates for a possible antagonist of vitamin B₆ was the compound "3-thio-4-deoxypyridoxine" (5-mercapto-4,6-dimethyl-3-pyridinemethanol, VIIa) in which the phenolic group of 4-deoxypyridoxine has been replaced by a mercapto group. The reaction of acetylacetone with 2-cyanoacetamide is known to give 4,6-dimethyl-2-hydroxynicotinonitrile (I)[4] and this served as a starting point for the total synthesis of the target compound. Basic hydrolysis of the nitrile I gave 4,6-dimethyl-2-hydroxynicotinic acid (IIa) which was easily esterified by a modified Fisher procedure to give ethyl 2-hydroxy-4,6-dimethylnicotinate (IIIa). Nitrations of the acid IIa and of the ester

(1) For paper I of this series see J. L. Greene, Jr., and J. A. Montgomery, *J. Med. Chem.*, **6**, 294 (1963).

(2) This work was supported by funds from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740.

(3) See footnote 3, ref. 1, and H. E. Skipper, J. R. Thomson, and F. M. Schabel, Jr., *Cancer Chemotherapy Rept.*, **29**, 63 (1963).

(4) J. Moir, *J. Chem. Soc.*, **81**, 105 (1902).