TABLE I

INTERCONVERSION REACTIONS

Alkyl group	Derivative	Yield, %	M.p. (b.p.), °C.	Analyses Calcd. C	Calcd. H	Calcd. B	Calcd. N	Found C	Found H	Found B	Found N
1-Propyl	Trimethylamine Borane	65		62.64	15.77	9.41	12.18	62.45	15.60	9.50	12.06
	Dihydrobenzoboradiazole	95	102–103								
	Borazole	88	108 (9 mm)	52.28	11.70	15.70	20.32	52.05	11.51	15.74	20.25
2-Propyl	Trimethylamine Borane	65		62.64	15.77	9.41	12.18	62.54	15.79	9.39	12.23
	Dihydrobenzoboradiazole	91	124–126								
	Borazole	79	(70/0.5)	52.28	11.70	15.70	20.32	51.95	11.62	15.60	20.51
1-Butyl	Trimethylamine Borane	64		65.14	15.62	8.38	10.86	64.88	15.38	8.23	10.83
	Dihydrobenzoboradiazole	85	66–67								
	Borazole	91	(110/0.6)	57.92	12.16	13.04	16.88	57.97	12.00	12.94	16.68
2-Butyl	Trimethylamine Borane	66		65.14	15.62	8.38	10.86	65.02	15.29	8.41	10.80
	Dihydrobenzoboradiazole	92	61–62								
	Borazole	85	(94/0.7)	57.92	12.16	13.04	16.88	57.90	11.91	12.90	16.72

spectra. The above table gives data concerning the transformations of four representative alkylboroxines.

$$RBH_2^- - NH_3^+ \xrightarrow[NH_4Cl]{NH_3} [RBH_2^- - NH_3^+] \longrightarrow 2H_2 + 1/3\ (^-RB{\equiv}NH)_3^+$$

The scope of these reactions are under investigation and will be reported later in greater detail.

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A TPN+ SPECIFIC GLYCEROL DEHYDROGENASE FROM LIVER*

Sir:

The DPN+ specific glycerol dehydrogenases of rat and swine liver have been described.[1] I wish to report here the occurrence of a TPN+ specific glycerol dehydrogenase which was found during the process of chromatography of the supernatant fraction of a rat liver sucrose (0.25 *M*) homogenate. This enzyme may be of general interest since it was found to oxidize tris-(hydroxymethyl)-aminomethane and other similar compounds used as biochemical buffers. A volume of 20 ml. of supernatant, containing about 200 mg. protein, which had been dialyzed against 5 m*M* tris phosphate, *p*H 8.0, was applied to a 1.1 × 30 cm. column of DEAE-cellulose,[2,3] and initially eluted with about 150 ml. of the same buffer to removed loosely bound proteins. The two glycerol dehydrogenases emerged as separate peaks of activity as shown in Fig. 1. The total activity of the TPN+ was about one-eighth that of the DPN+ enzyme. The purification of the enzyme during the chromatography was 40–50 fold.

The enzyme may be classed as a D-glyceraldehyde hydrogenase: the equilibrium of the reaction lies far toward the direction of glyceraldehyde reduction[1]; dihydroxyacetone was found to give less than one-fifth the activity obtained with DL-glyceraldeyde; and D-glyceraldeyde was found to be more active than the DL-form.

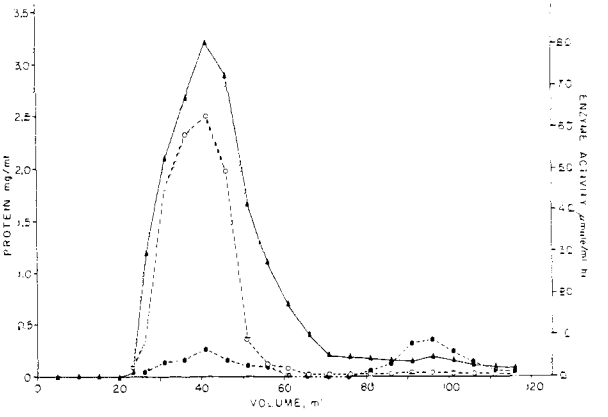

Fig. 1.—Separation of DPN+ and TPN+ D-glyceraldehyde hydrogenases on DEAE-cellulose. The reaction mixture contained 20 μmole of DL-glyceraldehyde, 4 μmole of triethanolamine *p*H 7.0, and 0.02 μmole DPNH or TPNH in a volume of 200 microliters. Incubation was 30 min. at 38°. Triangles represent protein (Lowry); open circles, enzyme activity with DPNH; closed circles, with TPNH.

The optimum *p*H for glyceraldehyde reduction was less than 7.0 with 0.1 *M* DL-glyceraldehyde, 0.02 *M* triethanolamine buffer, and 10^{-4} *M* TPNH. When the reaction was run in the opposite direction, at 0.1 *M* substrate concentration and at the optimum *p*H of 9.5 in triethanolamine buffer, glycerol was oxidized, but 2-amino-2-methyl-1, 3-propanediol (AMP_2) was oxidized at about twice the rate. Tris-(hydroxymethyl)-aminomethane and 2-amino-2-methyl-1-propanol (AMP_1) were oxidized at about two-thirds the rate of glycerol, and ethanol was not oxidized at a measurable rate. The K_m's at 38° for glycerol and AMP_2 were 0.63 *M* and 0.13 *M*, respectively. The K_m for TPN+ in the presence of 0.5 *M* AMP_2 was 1.7×10^{-4} *M*. The K_m for D-glyceraldehyde was 6.2×10^{-4} *M*. The enzyme was 95% inhibited by 10^{-5} *M p*-mercuribenzoate.

* These abbreviations are used: TPN+ and TPNH, oxidized and reduced forms of triphosphopyridine nucleotide, respectively; DPN oxidized diphosphopyridine nucleotide; DEAE-cellulose, diethylaminoethyl-cellulose.

This work was supported by grant C-4110 from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

(1) H. P. Wolf and F. Leuthardt, *Helv. Chim. Acta*, **36**, 1463 (1953).
(2) E. A. Peterson and H. A. Sober, THIS JOURNAL, **78** 751 (1956).
(3) H. A. Sober, F. J. Gutter, M. M. Wycoff and E. A. Peterson, *ibid.*, **78**, 756 (1956).

A description of the entire DEAE-cellulose chromatogram of the supernatant was presented earlier,[4] and will be reported in detail elsewhere.

(4) B. W. Moore, *Fed. Proc.*, **18**, 289 (1959).

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A GENERAL SYNTHESIS OF THE PENICILLINS

Sir:

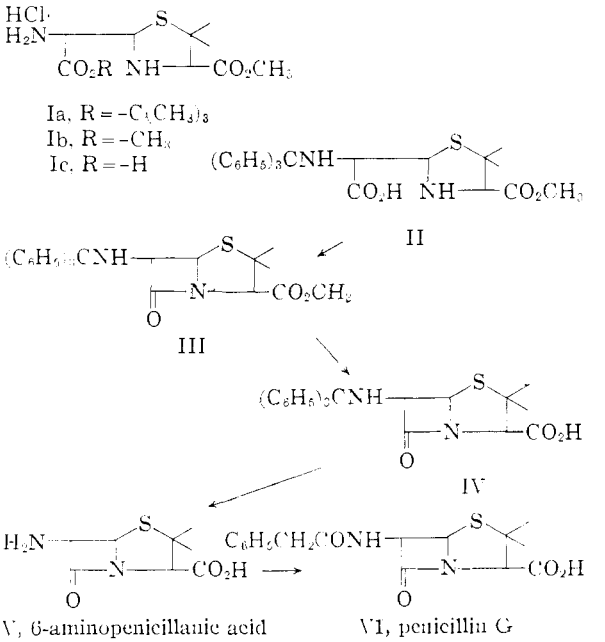
This Communication describes both a partial (from penicillin G) and a total general synthesis of the penicillins. The key intermediate in the two routes is 6-aminopenicillanic acid (V),[1] which we have acylated to form both "natural" penicillins and penicillins not obtainable directly by fermentation.[2]

In the total synthesis series, removal of the phthaloyl group from *t*-butyl D-α-4-carbomethoxy-5,5 - dimethyl - α - phthalimido - 2 - thiazolidineacetate[3] as described for the corresponding DL-isomer[4] produced *t*-butyl D-α-4-carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidineacetate hydrochloride (Ia), $C_{12}H_{25}N_2O_4SCl$, in 68% yield; m.p. 183–184° (dec.), $\alpha^{31}D$ + 91° (*c* 1.2 in methanol) [found: C, 45.65; H, 7.61; N, 8.31]. Cleavage of the *t*-butyl ester with hydrogen chloride led to a 92% yield of the dihydrochloride of D-α-4-carbomethoxy - 5,5 - dimethyl - α - amino - 2 - thiazolidineacetic acid (Ic), $C_9H_{18}N_2O_4SCl_2$; m.p. 94–97° (dec.), $\alpha^{29}D$ + 82° (*c* 0.5 in 6 *N* hydrochloric acid) [found: C, 33.72, H, 5.88; N, 8.93]. Trityl chloride and diethylamine[5] converted Ic into D-α - 4 - carbomethoxy - 5,5 - dimethyl - α - tritylamino-2-thiazolidineacetic acid (II). Treatment of II with N,N′-diisopropylcarbodiimide in dioxane–water, followed by chromatography over neutral alumina, afforded crystalline methyl 6-tritylaminopenicillanate (III) in 25% yield, $C_{28}H_{28}N_2O_3S$; m.p. 165–166°, $\alpha^{31}D$ + 96° (*c* 1.1 in *n*-butyl acetate), λ_{max}^{KBr} at 5.63(vs)μ [found: C, 71.29; H, 6.07; N, 5.73].

By the partial synthesis route, saponification of the α-methyl ester grouping of Ib (obtained from penicillin G[6]) with one equivalent of sodium hydroxide and tritylation[5] of this product formed II in 20% over-all yield as a non-crystalline solid, $C_{28}H_{30}N_2O_4S$; m.p. 87–89° (dec.), $\alpha^{31}D$ + 45° (*c* 1.3 in *n*-butyl acetate) [found: C, 68.74; H, 6.28; N, 5.55]. Cyclization of II with diisopropylcarbodiimide gave crystalline III, in 22% yield, m.p. 165–166°.

Compound III also was prepared in 25% overall yield by tritylation of natural 6-aminopenicillanic acid followed by esterification with diazomethane, m.p. 165–166°, undepressed upon admixture with the corresponding samples made by total synthesis and from penicillin G and having an identical infrared spectrum (KBr) and optical rotation.

The methyl ester of III was saponified selectively[7] to afford 6-tritylaminopenicillanic acid (IV) as the crystalline diethylamine salt in 17% yield, $C_{31}H_{37}N_3O_3S$; m.p. 166–168° (dec.), $\alpha^{29}D$ + 89° (*c* 1 in dioxane), λ_{max}^{KBr} at 5.66(vs)μ [found: C, 69.71; H, 7.00; N, 7.90). The salt responded to the quantitative hydroxylamine assay for penicillins.[8] Tritylation[5] of V gave the diethylamine salt of IV in 26% yield, m.p. 164–166° (dec.). Identity of samples of IV prepared from III and V, respectively, was established by undepressed mixed m.p., identical infrared spectra (KBr) and rotations.



Detritylation of IV with dilute hydrochloric acid gave 32% of crystalline 6-aminopenicillanic acid (V), $C_8H_{12}N_2O_3S$; m.p. 207–208° (dec.), [natural,[2] 208–209° (dec.)], undepressed mixed m.p. with natural, $\alpha^{31}D$ + 273° (*c* 1.2 in 0.1 *N* hydrochloric acid) [found: C, 44.43; H, 5.54; N, 12.86]. The infrared spectra (KBr) of natural and synthetic V were identical. Synthetic V was compared to natural V, in parallel determinations involving phenoxyacetylation followed by microbiological assay of the penicillin V formed, and shown to contain 107 ± 10% of 6-aminopenicillanic acid.

Acylation of V with phenylacetyl chloride in aqueous acetone containing sodium bicarbonate

(1) J. C. Sheehan, K. R. Henery-Logan and D. A. Johnson, THIS JOURNAL, **75**, 3292 (1953), footnote 2.

(2) In a Ciba Foundation Symposium held in London, England, in March, 1958, J. C. S. stated that ". . . we have prepared this compound [6-aminopenicillanic acid] via a totally synthetic route. . . . We have shown that one can acylate with various acid chlorides and obtain the corresponding penicillin" ("Amino Acids and Peptides with Antimetabolic Activity," G. E. W. Wolstenholme and C. M. O'Connor, Editors, J. A. Churchill Ltd., London, England, 1958, p. 258). More recently the isolation of 6-aminopenicillanic acid directly from penicillin fermentation broths has been reported by F. R. Batchelor, F. P. Doyle, J. H. C. Nayler and G. N. Rolinson, *Nature*, **183**, 257 (1959).

(3) J. C. Sheehan and K. R. Henery-Logan, THIS JOURNAL, **81**, 3089 (1959).

(4) J. C. Sheehan and P. A. Cruickshank, *ibid.*, **78**, 3677 (1956).

(5) L. Zervas and D. M. Theodoropoulos, *ibid.*, **78**, 1359 (1956).

(6) J. C. Sheehan and J. P. Ferris, *ibid.*, **81**, 2912 (1959).

(7) H. T. Clarke, J. R. Johnson and R. Robinson, Editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 94.

(8) J. H. Ford, *Anal. Chem.*, **19**, 1004 (1947).