addition of 6 *N* HCl and diluted with a fourfold volume of acetone. The crystals were collected by filtration and dried. They melted at 188–194° dec and weighed 4.7 g (73.0%). The analytical sample obtained by recrystallization from the same solvent melted at 197–199° and showed $[\alpha]D$ +150° (H_2O).

Anal. Calcd for $C_{18}H_{34}N_2O_6S \cdot HCl$: C, 48.80; H, 7.96; N, 6.32; S, 7.24. Found (corrected for 5.54% water): C, 48.58; H, 8.19; N, 6.04; S, 7.36.

Methyl N-(1′-Methyl-4′-*n*-butyl-L-prolyl)thiolincosaminide Hydrochloride (*cis* and *trans*) (9, R = *n*-C_4H_9).—A mixture of 2.0 g of **8** (R = *n*-C_4H_9), 2.0 ml of 37% formalin, 150 ml of methanol, and 500 mg of 10% Pd–C was shaken under 2.45 kg/cm² of H_2 for 3.5 hr. The catalyst was removed by filtration. Evaporation of the methanol yielded a partially crystalline residue which when assayed by tlc consisted chiefly of the *cis* and *trans* isomers of **9** (R = *n*-C_4H_9) in a ratio of about 3:1.

Separation of Isomers.—The above product was dissolved in a mixture of methanol–methylene chloride and 1.5 ml of triethylamine was added. To this solution was added 7 g of silica gel and the solvent was distilled under vacuum. This solid was sifted on top of a chromatographic column of 200 g of silica gel packed with a solvent mixture consisting of ethyl acetate, acetone, and water in a ratio of 8:5:1. The column was developed by eluting with the same solvent and 20-ml fractions were collected. Tlc of each fraction showed that fractions 31–38, 310 mg, were essentially pure *trans* isomer and 49–74, 326 mg, were essentially pure *cis* isomer. Fractions 39–48 consisted of a mixture of isomers which could be further separated by repeated chromatography. Each isomer was dissolved in a few drops of dilute HCl and the hydrochloride precipitated by addition of acetone. In this manner there was obtained 50 mg of *trans* **9** (R = C_4H_9), mp 135–137° dec, and about 150 mg of *cis* isomer, mp 105°, softening with further melting at 175–185° dec.

The *trans* isomer was recrystallized from the same solvent and then melted at 161–168° dec.

Anal. Calcd for $C_{19}H_{36}N_2O_6S \cdot HCl$: C, 49.93; H, 8.16; N, 6.13; S, 7.02. Found: C, 50.32; H, 7.98; N, 6.20; S, 6.67. N and S analysis corrected for 4.07% H_2O; C and H sample dried at 100°.

Similarly, recrystallization of the *cis* isomer gave a product melting at 194–198° dec.

Anal. Found (corrected for 4.34% water): C, 49.82; H, 8.20; N, 6.05; S, 6.65.

Methyl N-(1′-Ethyl-4′-*n*-butyl-L-prolyl)thiolincosaminide Hydrochloride (*cis* and *trans*) (10, R = *n*-C_4H_9).—A mixture of 2.0 g of **8** (R = *n*-C_4H_9), 1.5 ml of acetaldehyde, and 750mg of 10% Pd-C in 150 ml of methanol was shaken under 2.45 kg/cm² of H_2 for 5.5 hr. The catalyst was removed by filtration to give a residue consisting chiefly of *cis* and *trans* **10**, R = *n*-C_4H_9.

Separation of Isomers.—As described above, the mixture of isomers (2 g) was chromatographed over 200 g of silica gel, using for elution a solvent system of ethyl acetate–acetone–water (8:5:1). Fractions 33–42 appeared by tlc to be pure *trans* isomer and were combined. Fractions 47–64 were essentially pure *cis* isomer and were also combined. Fractions 43–48 were a mixture of isomers which could be purified by rechromatography. Each isomer was dissolved in a few drops of dilute HCl and the crystalline hydrochloride precipitated on dilution with a large volume of acetone.

The *trans* isomer fraction of 415 mg gave 340 mg (15.9%) of crystalline *trans* product, mp 144–151°. Recrystallization from dilute acetone raised the melting point to 148–151°.

Anal. Calcd for $C_{20}H_{38}N_2O_6S \cdot HCl$: C, 50.99; H, 8.35; N, 5.95; S, 6.81. Found (corrected for 4.08% water): C, 51.33; H, 8.68; N, 5.81; S, 6.57.

The *cis* isomer fraction of 645 mg afforded 300 mg (14.1%) of crystalline hydrochloride, mp 135–139°. Recrystallization from dilute acetone gave crystals, mp 134–138°.

Anal. Found (corrected for 3.30% water): C, 50.94; H, 8.54; N, 5.98; S, 6.50.

Methyl N-(1′-Ethyl-4′-*n*-hexyl-L-prolyl)-7-deoxy-7(S)-chlorothiolincosaminide (11, R = *n*-C_6H_{11}).—Thionyl chloride (1.4 ml) was added to a stirred suspension of methyl N-(1′-ethyl-4′-*n*-hexyl-L-prolyl)thiolincosaminide (1.4 g) in 28 ml of CCl_4. The reaction mixture was heated at reflux for 2 hr and evaporated to dryness under vacuum. Chloroform (100 ml) was added and then removed under vacuum. The $CHCl_3$ addition and evaporation were repeated two more times to ensure complete removal of residual $SOCl_2$. The solid residue was dissolved in 5 ml of ethanol, cooled in an ice bath, and the pH was adjusted to 10 by the addition of 1 *N* NaOH. Water (200 ml) was added and the basic solution was extracted well with $CHCl_3$. The extracts were dried and evaporated to give 1.0 g of crude product. This material was purified by chromatography over silica gel using MeOH–$CHCl_3$ (1:6) for elution. The fractions containing the desired material as determined by tlc were combined and evaporated. The residue was converted to its hydrochloride salt as described above.

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The 6-Deoxytetracyclines. IX. Imidomethylation

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Reaction of 6-demethyl-6-deoxytetracycline with N-hydroxymethylphthalimide gives 7-phthalimidomethyl-6-demethyl-6-deoxytetracycline. Reaction of other N-methylolimides is also described. 7-Citraconimidomethyl-6-demethyl-6-deoxytetracycline has an *in vitro* biological activity 12 times that of tetracycline.

The reaction of an aromatic compound with an N-hydroxymethylamide or an N-hydroxymethylimide to form an amidomethyl- or imidomethyl-substituted product has beeen known for many years as the Tscherniac–Einhorn reaction.[1] This reaction has recently been reviewed excellently by Zaugg and Martin[2a] as well as by other workers;[2b,c] however, the reaction does not seem to have enjoyed very wide synthetic import. No example of a natural product having been subjected to this reaction is reported by these reviewers, although it is clear that the general scope that it encompasses is broad.

Nitration[3] and halogenation[4] of 6-deoxytetracyclines

(1) J. Tscherniac, German Patent 134,979 (1902); *Chem. Zentr.*, II, 1084 (1902); A. Einhorn, J. Bischkopff, and B. Szelinski, *Ann.*, **343**, 223 (1905).

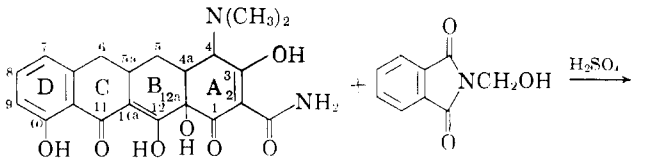
(2)(a) H. E. Zaugg and W. B. Martin, *Org. Reactions*, **14**, 52 (1965); (b) R. Schröter in Houben-Weyl "Methoden der Organischen Chemie," Vol. XI/1, 4th ed, G. Thieme, Stuttgart, 1957, pp 795–805; (c) H. Hellmann, *Angew. Chem.*, **69**, 463 (1957); H. Hellmann in "Newer Methods of Preparative Organic Chemistry," Vol. 2, W. Foerst, Ed., Academic Press Inc., New York, N. Y., 1963, pp 277–302.

(3)(a) J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens, *J. Am. Chem. Soc.*, **82**, 1003 (1960); (b) J. Petisi, J. L. Spencer, J. J. Hlavka, and J. H. Boothe, *J. Med. Pharm. Chem.*, **5**, 538 (1962).

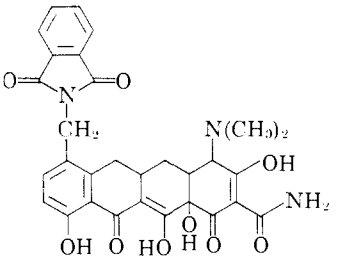
(4) J. J. Hlavka, A. Schneller, H. Krazinski, and J. H. Boothe, *J. Am. Chem. Soc.*, **84**, 1426 (1962); C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. Schach Von Wittenau, *ibid.*, **85**, 2643 (1963).

in strong acid have been described from these and other laboratories and lead to products which in many cases have favorably enhanced biological activity. Seeking then to find other areas of electrophilic substitution which would be adaptable to 6-deoxytetracyclines we subjected 6-demethyl-6-deoxytetracycline (I) to the conditions of the Tscherniac-Einhorn reaction. When I and N-hydroxymethylphthalimide were treated in concentrated sulfuric acid at room temperature for about 40 min a crude mixture was obtained (Scheme I)

SCHEME I



I



II

which showed a new spot on a paper chromatogram and phthalimide carbonyl bands in the infrared spectrum at 5.6 and 5.8 μ. Subjection of the mixture to liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth gave what appeared to be a single product, which was difficult to separate from excess starting material. This product had an *in vitro* biological activity 3.2 times that of tetracycline or a twofold increase over starting material. The biological data are summarized in Table I.

TABLE I
RELATIVE[a] ACTIVITIES OF IMIDOMETHYL DERIVATIVES

		In vivo[b]	
Compd	*In vitro*[a]	Oral	Iv
II	3.2	1	...
III	7.0	<0.06	<0.06
IV	2.3	0.5–1.0	0.25
V	12.0	<0.06	0.25
VI	0.382	<0.06	<0.25

[a] Determined against *Staphylococcus aureus* 209P by the method of E. Peleak and A. Dornbush [*Ann. N. Y. Acad. Sci.*, **51**, 218 (1940)]. [b] Determined against *Staphylococcus aureus* strain Smith in mice. [c] Tetracycline = 1.0.

The assignment of the position of substitution at the 7 position is based on radioactive labeling experiments, similar to those which were used to determine the structure of nitration[3] and halogenation[4] products. Thus, I with a tritium atom at 7[3b] was treated with N-methylolphthalimide and the crude reaction product subjected to paper chromatography. The spot corresponding to II was cut out and combusted, and the radioactivity was determined. There was no rise in radioactivity above background. As a control reaction, radioactive I was dissolved in sulfuric acid at room temperature and isolated after 40 min. Approximately half the radioactivity had been lost by exchange but the amount retained still exceeded the background counts and II by a factor of about 10.

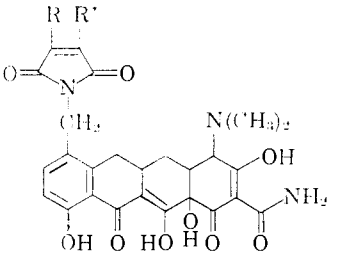
Time studies determined that unlike halogenation and nitration this reaction did not proceed well at ice-bath temperatures, and about 40 min at room temperature was required. A study of other acids also showed that concentrated sulfuric acid was the best agent for effecting this condensation, although methanesulfonic and anhydrous HF also gave partial success. Acids such as trifluoroacetic and polyphosphoric failed, however, as did less concentrated solutions of H_2SO_4 with water or acetic acid.

Zaugg and Schaefer[5] have also reported that bromomethylphthalimide reacts with phenol without the aid of zinc chloride or other Lewis acid to give aromatic substitution. However, bromomethylphthalimide and I in refluxing dimethoxyethane did not give II, although it was clear that reaction had taken place from paper chromatographic evidence, since no starting material was left.

Attempts to "deblock" II to the aminomethyl derivative by reaction with hydrazine[6,7] gave reaction at the 11,12-dicarbonyl system[8] as shown by ultraviolet spectra, and the use of *n*-butylamine in refluxing methanol[9] caused decomposition. Zaugg and Martin[10] have stated that *p*-phthalimidomethylphenol could not be converted to *p*-hydroxybenzylamine either with hydrazine or by hydrolytic means without destruction of the molecule.

Conversion of II to 7-methyl-6-demethyl-6-deoxytetracycline could not be achieved and failed by the methods tried. Although hydrogenolysis of acyl benzylamines is extremely difficult[11] and is rarely successful, reaction with sodium and liquid ammonia has been reported to be effective.[12] However, II was destroyed by the reaction conditions, the BCD ring chromophore being attacked.

Since II had substantial biological activity (Table I) preparation of simpler analogs seemed of interest. Thus N-hydroxymethylmaleimide[13] reacted similarly with I to give III (by analogy with II) which could be purified by liquid–liquid partition chromatography.



III, R = R' = H
IV, R = R' = CH_3
V, R = H; R' = CH,

(5) H. E. Zaugg and D. Schaefer, *J. Org. Chem.*, **28**, 2925 (1963).
(6) H. Ing and R. H. F. Manske, *J. Chem. Soc.*, 2348 (1926).
(7) J. Sheehan and V. S. Frank, *J. Am. Chem. Soc.*, **71**, 1856 (1949).
(8) U. Valcavi, G. Campanella, and N. Pacini, *Gazz. Chim. Ital.*, **93**, 916 (1963).
(9) L. Goldman and A. W. Marsico, *J. Med. Chem.*, **6**, 413 (1963); F. S. Spring and J. C. Woods, *Nature*, **158**, 754 (1946).
(10) H. E. Zaugg and W. B. Martin, *Org. Reactions*, **14**, 123 (1965).
(11) W. D. Schaeffer and A. C. McKinnis, U. S. Patent 2,988,576 (June 13, 1961).
(12) S. Sugasawa and T. Fujii, *Chem. Pharm. Bull.* (Tokyo), **6**, 587 (1958).
(13) P. O. Tawney, R. D. Snyder, R. P. Conger, K. A. Leibbrand, C. H. Schieler, and A. R. Williams, *J. Org. Chem.*, **26**, 15 (1961).

Table I shows that this compound had an *in vitro* activity seven times that of tetracycline.

In order to define more clearly the parameters for biological activity which seem acutely sensitive to subtle chemical differences at the 7 position, alkylated maleimides were investigated. N-Hydroxymethyl-2,3-dimethylmaleimide reacted with I to give a mixture of products from which two components could be separated by liquid–liquid partition chromatography. One of these was antibacterially active and was assigned structure IV. The other compound which had physical properties very similar to IV was not biologically active. An nmr spectrum [in $(D_3C)_2SO$] clearly showed that the material was a 7,9-disubstituted derivative. Signals at 420, 272, and 115 cps were in a ratio of 1:4:12 corresponding to the aromatic, benzylic and C-methyl protons.

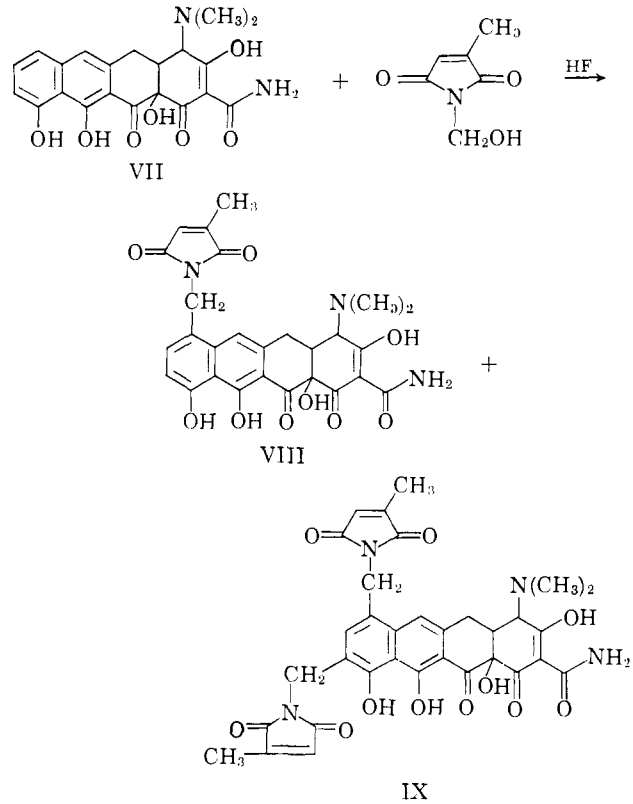
To complete the investigation, a synthesis of N-hydroxymethyl-2-methylmaleimide (N-methylolcitraconimide) was effected from the difficultly made citraconimide synthesized by a modification of the literature procedure.[14] The 7-citraconimidomethyl-6-demethyl-6-deoxytetracycline (V) which resulted from the ensuing condensation, after purification, had an *in vitro* activity 12 times that of tetracycline, the most potent known such derivative. Its activity compared favorably with that of novobiocin against gram-positive organisms.

Reduction of III gave 7-succinimidomethyl-6-demethyl-6-deoxytetracycline (VI) which had only about 4% the *in vitro* activity of III. Reaction of N-hydroxymethylsuccinimide and I failed.

The generality of the reaction was demonstrated by extending the reaction to the anhydrotetracycline series. In this case, however, anhydrous HF proved to be a more effective reaction medium since concentrated H_2SO_4 caused extensive sulfonation of the starting material. When 6-demethylanhydrotetracycline (VII) was treated with N-methylolcitraconimide in liquid anhydrous HF, two products were isolated (Scheme II). One of these products had a substantially enhanced *in vitro* biological activity (0.4 times that of tetracycline) compared with the activity of starting material (0.2 times that of tetracycline). This represents the first example of an anhydrotetracycline having its activity favorably enhanced by chemical modification. This compound was assigned the 7-substituted structure VIII on the basis of its activity. The other compound obtained, which was biologically inactive, was identified as the 7,9-dicitraconimido structure IX on the basis of its nmr spectrum in $(D_3C)_2SO$. The C_8 aromatic proton at 435 cps was unsplit as was the C_6 proton at 423 cps. The aromatic protons, the vinyl protons at 400 cps, the benzylic protons at 287 and 279 cps, and the C-methyl protons at 120 and 122 cps were in the properly integrated ratio.

From the data presented one can make some empirical observations concerning the interesting biological activities of these compounds. First, the double bond in the imide group appears to enhance activity since VI is comparatively inactive. Second, the steric environment around the double bond is important since II and IV are disubstituted and are far less active than

SCHEME II



III and V. Maleimide and citraconimide are known to add SH groups very rapidly[15] which may be of importance in the antibacterial activity or decreased *in vivo* activity of these compounds.

Experimental Section

Descending paper chromatography was carried out on Whatman No. 1 paper buffered with 0.2 *M* phosphate buffer, using a system 1-butanol–phosphate buffer pH 2.0 (2:1). Liquid–liquid partition chromatography[16] was carried out on neutral (acid-washed) diatomaceous earth (Celite). Melting points were determined on a Thomas–Hoover apparatus and are corrected. Nmr spectra were determined on a Varian A-60 spectrometer using Me_4Si as the internal standard. Analyses were performed by Mr. L. Brancone and staff or by the Schwartzkopf Microanalytical Laboratory, Woodside, N. Y. Optical rotations and spectra were determined by Mr. W. Fulmor and staff.

N-Hydroxymethyl-2,3-dimethylmaleimide.—To a mixture of 1.25 g (10 mmoles) of 2,3-dimethylmaleimide[17] and 0.81 ml (11 mmoles) of 40% aqueous formaldehyde was added 0.03 ml of 5% aqueous NaOH (*ca.* pH 7.5). The solid dissolved, and the solution was allowed to stand at room temperature for 3 hr, then evaporated to an oil *in vacuo*. The flask was placed in an acetone–Dry Ice bath whereupon the oil froze to a glass. Upon warming to room temperature, the glass crystallized. The yield was quantitative. The material was sublimed at 50° (0.1 mm) for analysis, mp 48–50°.

Anal. Calcd for $C_7H_9NO_3$: C, 54.2; H, 5.9; N, 9.0. Found: C, 54.5; H, 5.8; N, 9.0.

N-Hydroxymethylcitraconimide.—The material was prepared from citraconimide[14] as described above. The compound was recrystallized from benzene–hexane and sublimed at 60° (0.1 mm) for analysis, mp 68°.

Anal. Calcd for $C_6H_7NO_3$: N, 9.93. Found: N, 10.07.

(14) P. M. Brown, D. B. Spiers, and M. Whalley, *J. Chem. Soc.*, 2882 (1957).

(15) E. Friedmann, *et al.*, *Brit. J. Pharmacol.*, **4**, 105 (1949); *Biochem. Biophys. Acta*, **9**, 61 (1952); D. H. Marrian, E. Friedmann, and J. L. Ward, *Biochem. J.*, **54**, 65 (1953).

(16) M. J. Weiss, R. E. Schaub, G. R. Allen, Jr., J. F. Poletto, C. Pidacks, R. B. Conrow, and C. J. Coscia, *Tetrahedron*, **20**, 357 (1964).

(17) R. Otto and H. Beckurts, *Ber.*, **18**, 835 (1885).

TABLE II

Derivative of 6-DMDOTC[f]	R_f[a]	$[\alpha]^{25}D$, deg (c, methoxyethanol)	Partition chromatography data[b]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
7-Phthalimidomethyl-	0.9	+4.3 (0.464)	H–EA–ME–W (1:5:2:1) HBV 0.4–0.6[c]	$C_{30}H_{27}N_3O_9$	62.82	4.74	7.33	62.86	4.96	6.59
7-Maleimidomethyl-	0.68	−38 (0.527)	C–D–W (6:5:1) HBV 3.2–5.0	$C_{26}H_{25}N_3O_9 \cdot C_4H_4O_4$[d]	58.91	5.44	6.87	59.62	5.52	6.66
7-Citraconimidomethyl-	0.79	−32 (0.500)	C–D–W (7:4:1) HBV 6.5–9.0	$C_{27}H_{27}N_3O_9$	60.33	5.06	7.82	60.43	5.52	7.78
7-(2,3-Dimethylmaleimido)-methyl-	0.89	−1.97 (0.507)	H–EA–ME–W (65:35: 20:10) HBV 7.4–10.0	$C_{28}H_{29}N_3O_8$	60.97	5.30	7.62	60.71	5.41	7.14
7,9-Di[(2,3-dimethylmaleimidomethyl)]-	0.93	−18.6 (0.537)	H–EA–ME–W (65:35: 20:10) HBV 11.0–18.0	$C_{36}H_{36}N_4O_{11} \cdot H_2O$	59.48	5.42	7.93	59.96	5.67	7.56
7-Succinimidomethyl-	0.49			$C_{26}H_{27}N_3O_9 \cdot 0.5H_2O$	58.42	5.28	7.86	58.33	6.21	7.49
7-Citraconimidomethyl-6-demethylanhydrotetracycline	0.83	+131 (0.517)	H–EA–ME–W (75:25: 17:6) HBV 2.6–3.8	$C_{27}H_{25}N_3O_9 \cdot 0.5H_2O$[e]	59.60	4.81	7.72	60.03	5.29	7.56

[a] R_f values are for the system 1-butanol–phosphate buffer, pH 2.0. [b] H = heptane, EA = ethyl acetate, ME = methoxyethanol, W = water, C = cyclohexane, D = *p*-dioxane. [c] HBV = hold-back volume (column solvent retention). [d] Solvent determined by nmr. [e] Solvent determined by vpc. [f] 6-Demethyl-6-deoxytetracycline.

General Method for Preparation of the Substitution Products. 7-Maleimidomethyl-6-demethyl-6-deoxytetracycline.—A solution of 453 mg (1.0 mmole) of 6-demethyl-6-deoxytetracycline hydrochloride in 5 ml of 96% H_2SO_4 at room temperature was treated with 190 mg (1.5 mmoles) of N-hydroxymethylmaleimide.[13] The solution was stirred at room temperature for 35 min, then poured into 150 ml of dry ether. The precipitate was filtered off, washed well with dry ether, and dried, 500 mg. The precipitate was dissolved in 18 ml of water and the pH was adjusted to 5.0 with 1 *N* aqueous NaOH. The precipitated material was filtered off, washed with two 1-ml portions of water, and dried *in vacuo*, 305 mg. The material was purified by liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth as indicated in Table II. A column was packed with 65 g of diatomaceous earth, moistened with 32.5 ml of the aqueous layer of a mixture of cyclohexane–*p*-dioxane–water (6:5:1). The sample (85 mg) was dissolved in 5 ml of aqueous phase and packed with 10 g of diatomaceous earth at the top of the column. Product (21 mg) was eluted in the fourth and fifth hold-back volumes of the column as the column was developed with upper phase.

7-Succinimidomethyl-6-demethyl-6-deoxytetracycline.—A solution of 315 mg (0.6 mmole) of chromatographically pure 7-maleimidomethyl-6-demethyl-6-deoxytetracycline in 6 ml of methoxyethanol and 100 mg of PtO_2 was hydrogenated at atmospheric pressure and room temperature. Uptake (32 ml, theory 34 ml) was complete in 1.5 hr. The catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was triturated with anhydrous ether, filtered off, and dried, 260 mg.

Reaction of 7-^{3}H-6-Demethyl-6-deoxytetracycline with N-Hydroxymethylphthalimide.—A solution of 7-^{3}H-6-demethyl-6-deoxytetracycline hydrochloride (226 mg, 0.5 mmole) in 3.3 ml of concentrated H_2SO_4 was treated at room temperature with 133 mg (0.75 mmole) of N-hydroxymethylphthalimide. The solution was stirred at room temperature for 40 min then slowly poured into 20 ml of dry ether. The precipitate was filtered off, washed well with dry ether, and dried, 336 mg. The crude material (15-μg load) was run on a paper chromatogram and the solvent front was allowed to run off the sheet to completely separate the product from any remaining starting material. A control experiment with 7-^{3}H-6-demethyl-6-deoxytetracycline was run to determine the extent of exchange and a comparison with product and background made. Areas corresponding to starting material and product were cut out and combusted, and the radioactivity was determined (Table III).

TABLE III

Compd	Counts/min	Dpm
II	122	5,700
	122	5,985
Control	925	65,687
7-^{3}H-6-DMDOTC[a]	1447	75,065
Background	59	2,762
	122	5,985

[a] 7-Tritio-6-demethyl-6-deoxytetracycline.

7-Citraconimidomethyl-6-demethylanhydrotetracycline and 7,9-Dicitraconimidomethyl-6-demethylanhydrotetracycline.—A solution of 412 mg (1.0 mmole) of 6-demethylanhydrotetracycline, neutral in 10 ml of anhydrous liquid HF in a polyethylene vessel in an ice bath, was treated with 155 mg (1.10 mmoles) of N-methyloleitraconimide. The solution was stirred in the cold for 50 min, then the HF was removed by passing N_2 through the reaction mixture. The residue was dissolved in 40 ml of water and brought to pH 5.0 with 15% aqueous NaOH. The yellow precipitate was filtered off, washed well with water, and dried, 535 mg.

A 150-mg portion of the above crude material was subjected to liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth, using a solvent system heptane–ethyl acetate–methoxyethanol–water (75:25:17:6). The monosubstituted derivative was obtained in hold-back volumes 2.6-

3.8 (26 mg) and the disubstituted derivative (as shown by nmr) (see discussion) was obtained in hold-back volumes 8.2–12.7 (18 mg).

Acknowledgments.—The authors are indebted to Mr. A. Dornbush for the *in vitro* testing, Mr. G. Redin for the *in vivo* results, Dr. A. Tomcufcik for helpful suggestions, and Dr. R. Kelly for the radioactive labeling determinations.

Semisynthetic Penicillins. IV. Pyridylmethylpenicillins and Related Cephalosporins[1]

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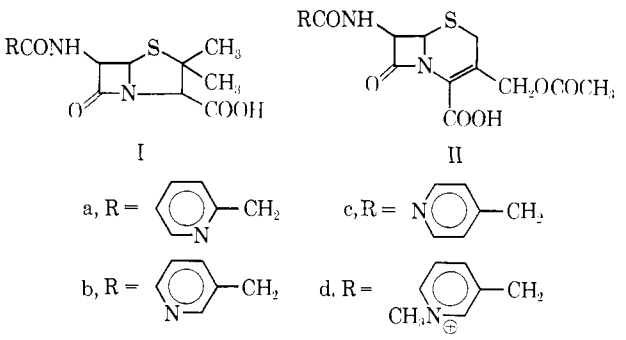
The preparation of the semisynthetic pyridylmethylpenicillins Ia–d and the related cephalosporins IIa–c is described. The *in vitro* antibacterial properties of these substances are reported. Some of them show promising broad-spectrum activity.

The work described in this paper was undertaken as part of a program designed to prepare, by chemical modification of 6-aminopenicillanic acid[2] and 7-aminocephalosporanic acid,[3] semisynthetic antibiotics with useful activity against both gram-positive and gram-negative bacteria. In the penicillin series,[4] benzylpenicillin (penicillin G) has excellent *in vitro* and *in vivo* activity against many gram-positive bacteria. However, it has only modest activity against gram-negative organisms and has rarely proved clinically useful against them. Phenoxymethylpenicillin (penicillin V) has poorer gram-negative activity than benzylpenicillin. The same is true of the sterically encumbered arylpenicillins, *e.g.*, methecillin (2,6-dimethoxyphenylpenicillin) and oxacillin (5-methyl-3-phenyl-4-isoxazolylpenicillin) which were designed to have resistance to staphylococcal penicillin lactamase. The only penicillin in clinical use with notable gram-negative activity is ampicillin, the D-α-amino derivative of benzylpenicillin. Another amino derivative of benzylpenicillin, *p*-aminobenzylpenicillin, has also been reported to have slightly enhanced *in vitro* gram-negative activity.[5] These data suggested that good gram-negative activity might be expected when the arylmethyl side chain of benzylpenicillin was retained and a basic site was incorporated into the molecule.[6] The pyridylmethylpenicillins fulfill these structural requirements. Scant data are available about structure–activity relationships in the cephalosporin series, but it has been suggested that the cephalosporin system is more amenable than the penicillin system to the development of derivatives which have gram-negative activity.[7]

We therefore decided to prepare the three isomeric pyridylmethylpenicillins[8,9] and the corresponding cephalosporins.



The penicillins Ia–c and the cephalosporins IIa–c were prepared by condensing the appropriate pyridineacetic acids with 6-aminopenicillanic acid or 7-aminocephalosporanic acid. The quaternary derivative Id was obtained by treating Ib with methyl iodide. The general method of preparing semisynthetic penicillins and cephalospirins by acylation of the appropriate nucleus is well established,[10] but its application in the present case required considerable departure from the customary techniques. The pyridineacetic acids or their hydrochlorides were converted to their acid chloride hydrochlorides.[11] The use of this technique for activating the pyridineacetic acids[9] was particularly advantageous in the case of the 2 and 4 isomers since it avoided the necessity of handling these compounds in their unstable zwitterionic form.[12] The acid chlorides were coupled with 6-aminopenicillanic or 7-

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(3) (a) B. Loder, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 408 (1961); (b) R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Am. Chem. Soc.*, **84**, 3400 (1962).

(4) An excellent review of the more important penicillins is given by J. O. Klein and M. Finland, *New Engl. J. Med.*, **269**, 1019, 1074, 1129 (1963).

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(6) (a) A recent report by W. J. Leanza, B. G. Christensen, E. F. Rogers, and A. A. Patchett, *Nature*, **207**, 1395 (1965), that *p*-guanidobenzylpenicillin has good *in vivo* activity against the gram-negative organism *Salmonella* is in agreement with this view. (b) R. Knox, *ibid.*, **192**, 492 (1961), considers other possible determinants of gram-negative activity.

(7) R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, *J. Am. Chem. Soc.*, **84**, 3401 (1962).

(8) (a) An unsuccessful attempt to prepare two of the pyridylmethylpencillins by biosynthesis has been reported by R. G. Jones, Q. F. Soper, O. H. Behrens, and J. W. Corse, *ibid.*, **70**, 2843 (1948). (b) Since the completion of this work, C. Hansch and E. W. Deutsch, *J. Med. Chem.*, **8**, 705 (1965), have suggested, on the basis of sequential analysis, that the pyridylmethylpenicillins might have good antibacterial activity.

(9) Since the completion of this work, successful use of the mixed anhydride and carbodiimide coupling procedures for the preparation of Ib and of some α-substituted derivatives of Ia and Ib has been claimed by L. C. Cheney and J. C. Godfrey, U. S. Patent 3,202,653 (1965).

(10) See, for example, J. R. E. Hoover, A. W. Chow, R. J. Stedman, N. M. Hall, H. S. Greenberg, M. M. Dolan, and R. J. Ferlauto, *J. Med. Chem.*, **7**, 245 (1964).

(11) The conditions used (PCl_5–AcCl) have been employed for converting amino acids to their acid chloride hydrochlorides. See, for example, H. Zinner and G. Brossmann, *J. Prakt. Chem.*, **5**, 91 (1957).

(12) W. von E. Doering and V. Z. Pasternak, *J. Am. Chem. Soc.*, **72**, 143 (1950).