which are essentially superimposable on those of the corresponding 6-methylated tetracyclines, showed that the Rings B–C–D chromophore was not changed,[7,8] leaving carbons 5a and 6 as possible points of difference.

R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, THIS JOURNAL, **75**, 5455 (1953).

(8) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **76**, 3568 (1954).

CHEMICAL PROCESS IMPROVEMENT DEPT. LEDERLE LABORATORIES DIVISION AMERICAN CYANAMID COMPANY PEARL RIVER, NEW YORK

J. R. D. MCCORMICK
NEWELL O. SJOLANDER
URSULA HIRSCH
ELMER R. JENSEN
ALBERT P. DOERSCHUK

RECEIVED AUGUST 1, 1957

DEMETHYLTETRACYCLINES. STRUCTURE STUDIES

Sir:

The isolation and characterization of a new series of highly active tetracycline antibiotics has been reported recently.[1] In this communication degradation studies are presented showing that the parent compound of this series is 6-demethyltetracycline.[2]

In a preliminary experiment 7-chloro-6-demethyltetracycline (I) was distilled with a highly active zinc dust[3] giving naphthacene ($\lambda_{max.}^{CHCl_3}$, 420 mμ, 446 mμ, 477 mμ). Under the same conditions chlorotetracycline gave a distillate whose ultraviolet spectrum [$\lambda_{max.}^{CHCl_3}$, 425 mμ (broad), 452 mμ (broad), 476 mμ, 485 mμ] was indicative of a mixture of naphthacene and 5-methylnaphthacene. These data coupled with the analytical results[1] indicating that these new antibiotics had one less carbon atom than the known tetracyclines[4] suggested that the C.6 methyl group of the tetracycline nucleus was missing in the new series. This was confirmed immediately by numerous Kuhn–Roth determinations on I and related compounds, all of which gave zero values. In this determination the familar tetracyclines all gave appreciable C-methyl values.[4b,5]

That the structure of I was the same as chlorotetracycline in all other respects[6] was shown by several reactions. Heating in concentrated hydrochloric acid aromatized the C ring of I giving an anhydro compound (II) in good yield, $[\alpha]^{25}D$ +105° (0.467% in methyl cellosolve); m.p. 205–210° (dec.), (*Anal.* Calcd. for $C_{21}H_{19}N_2O_7Cl$: C, 56.44; H, 4.29; N, 6.27; Cl, 7.94. Found: C, 56.39; H, 4.60; N, 5.83; Cl, 7.81; *C-methyl, 0.0*). This was considered to be good evidence for an hydroxyl group on C.6 and a hydrogen atom on C.5a. The ultraviolet absorption spectrum of II [$\lambda_{max.}^{0.1\,N\,HCl}$, 223 mμ (ε 30,200), 272 mμ (ε 52,500), 430 mμ (ε 8,050)] was quite similar to the known anhydrotetracyclines[7] but exhibited a hypsochromic shift characteristic of an aromatic compound having one less alkyl substituent.[8] The acid stability of II eliminated the possibility of C.5 bearing an hydroxyl group.[4b]

That I and the known tetracyclines have the same A ring, was shown by oxidation of I in *N* NaOH with oxygen to give dimethylamine and 3,4 - dihydroxy - 2,5 - dioxocyclopentane - 1 - carboxamide[9a] (III) identical with the compound obtained similarly from tetracycline and chlorotetracycline.[7b] From the same reaction was obtained a new compound (IV), $[\alpha]^{25}D$ +2.5° (2.00%, methanol), m.p. 194–195° (*Anal.* Calcd. for $C_{13}H_{11}O_7Cl$: C, 49.62; H, 3.52; Cl, 11.27. Found: C, 49.88; H, 3.80; Cl, 11.26; *C-methyl, 0.0*). On treatment of IV with diazomethane, a methoxydimethyl ester (V) was formed, m.p. 119.5–120.5° (*Anal.* Calcd. for $C_{16}H_{17}O_7Cl$: C, 53.86; H, 4.80; Cl, 9.94; CH_3O, 26.10. Found: C, 53.59; H, 5.08; Cl, 10.12; CH_3O, 26.66). Compound V was hydrolyzed to a methoxydiacid (VI), m.p. 210–217° (*Anal.* Calcd. for $C_{14}H_{13}O_7Cl$: C, 51.15; H, 3.98; Cl, 10.79. Found: C, 50.82; H, 4.09; Cl, 10.9) which was converted to a methoxy anhydride (VII), m.p. 242.5–243° (*Anal.* Calcd. for $C_{14}H_{11}O_6Cl$: C, 54.12; H, 3.57; Cl, 11.41. Found: C, 54.16; H, 3.82; Cl, 11.56). The infrared absorption spectrum of this latter compound had bands typical of a glutaric anhydride. Compounds IV–VII had ultraviolet and infrared absorption spectra characteristic of phthalides. Since acid permanganate oxidation of IV gave tricarballylic acid[9b] in good yield, it was postulated that IV is β-(4-chloro-7-hydroxyphthalide-3)-glutaric acid, corresponding to the 3-methyl compound[9] obtained in similar fashion from chlorotetracycline[7b] thus indicating that the B rings of both compounds must be the same. With the isolation of dimethylamine, III and IV the 21 carbons and 2 nitrogens in I were accounted for.

Proof that the D rings of I and of chlorotetracycline were the same and also confirmation of the tertiary nature of C.6 in I was obtained by a series of reactions used in a degradation of chlorotetracycline.[4a] Reduction of I with zinc dust and acetic acid yielded a dedimethylaminodeoxy compound (VIII), m.p. 212–217° (dec.) (*Anal.* Calcd. for $C_{19}H_{16}O_7NCl$: C, 56.23; H, 3.97; N, 3.45; Cl, 8.74. Found: C, 56.20; H, 3.66; N, 3.80; Cl,

(1) J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen and A. P. Doerschuk, THIS JOURNAL, **79**, 4561 (1957).

(2) The experiments reported herein were carried out on 7-chloro-6-demethyltetracycline. Its relationship to the parent compound and also to the 4-*epi*-isomers has been established in reference 1.

(3) (a) F. Kögl and W. B. Deijs, *Ann.*, **515**, 19 (1935). (b) We are indebted to Dr. J. B. Patrick for recommending this preparation.

(4) (a) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, THIS JOURNAL, **76**, 3568 (1954); (b) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **75**, 5455 (1953); (c) J. H. Boothe, J. Morton, II, J. P. Petisi, R. G. Wilkinson and J. H. Williams, *ibid.*, **75**, 4621 (1953).

(5) Unreported analyses from These Laboratories; for example, chlorotetracycline hydrochloride ($C_{22}H_{24}N_2O_8Cl_2$): Calcd. for 1 C-methyl, 2.92; Found: 1.93, 1.81, 1.91.

(6) The configurations about the five asymmetric centers in I and chlorotetracycline have not been related. However, it seems probable that they are the same in view of the high antibiotic activity of I and the strict configurational requirements for activity already demonstrated. *Cf.* A. P. Doerschuk, B. A. Bitler and J. R. D. McCormick, THIS JOURNAL, **77**, 4687 (1955), and footnote 3 of reference 1.

(7) (a) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, *ibid.*, **74**, 4981 (1952). (b) J. H. Boothe, J. Morton, II, J. P. Petisi, R. G. Wilkinson, and J. H. Williams, "Antibiotics Annual 1953–1954," Welch and Martí-Ibañez, Medical Encyclopedia, Inc., New York, N. Y., p. 46.

(8) F. Korte, *Angew. Chem.*, **63**, 375 (1951).

(9) (a) C. W. Waller, B. L. Hutchings, C. F. Wolf, R. W. Broschard A. A. Goldman and J. H. Williams, THIS JOURNAL, **74**, 4978 (1952); (b) B. L. Hutchings, C. W. Waller, S. Gordon, R. W. Broschard, C. F. Wolf, A. A. Goldman and J. H. Williams, *ibid.*, **74**, 3710 (1952).

8.87), which was isomerized with alkali. The resulting iso-compound, without purification, was pyrolyzed giving a small yield of 4-chloro-7-hydroxyphthalide, m.p. 155–157°, which was identified by comparison with a known sample prepared by an unambiguous synthesis reported by Boothe, *et al.*, in an accompanying communication.[10]

(10) J. H. Boothe, A. Green, J. P. Petisi, R. G. Wilkinson and C. W. Waller, THIS JOURNAL, **79**, 4564 (1957).

RESEARCH DIVISION — J. S. WEBB
ORGANIC CHEMICAL — R. W. BROSCHARD
RESEARCH SECTION — D. B. COSULICH
AMERICAN CYANAMID COMPANY — W. J. STEIN
PEARL RIVER, NEW YORK — C. F. WOLF

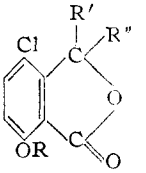
RECEIVED AUGUST 1, 1957

DEMETHYLTETRACYCLINES. SYNTHESIS OF A DEGRADATION PRODUCT

Sir:

In an accompanying communication[1] there is described a new series of antibiotics closely related to the tetracyclines, both in antibacterial activity and in structure. In a second accompanying communication[2] evidence has been presented that these new antibiotics differ from the parent compounds only in that they lack a methyl group at the 6-position of the tetracycline nucleus.

We now wish to report the synthesis of a degradation product which proves that the arrangement of substituents in the D ring and in portions of the C ring of demethylchlorotetracycline is the same as in chlorotetracycline except for the C-6 methyl group. This compound is 4-chloro-7-hydroxyphthalide (R = R′ = R″ = H) which is analogous to 4-chloro-7-hydroxy-3-methylphthalide (R = R′ = H, R″ = CH_3), obtained by the same degradative route from chlorotetracycline.[3]



The starting point for the synthesis is 4-chloro-3-hydroxy-7-methoxy-3-methylphthalide (R = R′ = CH_3, R″ = OH) whose synthesis has already been reported from this laboratory.[4] This compound was oxidized with potassium permanganate in 0.5 *N* sodium hydroxide at 90° for one hour to yield the 4-chloro-3-hydroxy-7-methoxyphthalide-3-carboxylic acid (R = CH_3, R′ = OH, R″ = COOH) in 67% yield. This compound has been isolated as a degradation product of chlorotetracycline and was named there as the tautomeric keto-acid, 6-chloro-3-methoxyphthalonic acid.[5] The reduction of this compound with sodium borohydride in *N* sodium hydroxide yielded 4-chloro-7-methoxyphthalide-3-carboxylic acid (R = CH_3, R′ = H, R″ = COOH) in 90% yield; m.p. 175–176° with effervescence; $\lambda_{max}^{0.1\,N\,HCl}$ 216 mμ (ε 32,200); 240 mμ (ε 8,240); 313 mμ (ε 5,220). $\lambda_{max}^{0.1\,N\,NaOH}$ (after standing one hour)[6] 214 mμ (ε 31,500); 285 mμ (ε 2,190).

Anal. Calcd. for $C_{10}H_7O_5Cl$: C, 49.5; H, 2.9; Cl, 14.6. Found: C, 49.5; H, 3.2; Cl, 14.8.

The phthalidecarboxylic acid was then decarboxylated by heating 5–10 minutes just above its melting point to yield 4-chloro-7-methoxy-phthalide (R = CH_3, R′ = R″ = H) which was sublimed at 175° (760 mm.); yield, 70%; m.p. 167–168° $\lambda_{max}^{0.1\,N\,HCl}$ 215 mμ (ε 37,300); 236 mμ (ε 8,830); 308 mμ (ε 4,560); $\lambda_{max}^{0.1\,N\,NaOH}$ (after standing one hour)[6] 286 mμ (ε 2,190).

Anal. Calcd. for $C_9H_7O_3Cl$: C, 54.4; H, 3.6; Cl, 17.9. Found: C, 54.8; H, 3.7; Cl, 17.7.

The methyl ether was cleaved by refluxing in 48% hydrobromic acid for 2.5 hours. The product, 4-chloro-7-hydroxyphthalide (R = R′ = R″ = H), crystallized from the hydrobromic acid on cooling in 70% yield and was then sublimed at 100° (15–20 mm.). The m.p. was 158–159° and there was no depression upon admixture with the degradation product.[2] The ultraviolet and infrared spectra were identical; $\lambda_{max}^{0.1\,N\,HCl}$ 235 mμ (ε 8,400); 308 mμ (ε 4,150); $\lambda_{max}^{0.1\,N\,NaOH}$ 254 mμ (ε 8,400); 343 mμ (ε 6,180).

Anal. Calcd. for $C_8H_5O_3Cl$: C, 52.1; H, 2.7; Cl, 19.2. Found: C, 52.2; H, 3.2; Cl, 19.0.

(1) J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen and A. P. Doerschuk, THIS JOURNAL, **79**, 4561 (1957).

(2) J. S. Webb, R. W. Broschard, D. B. Cosulich, W. J. Stein, and C. F. Wolf, *ibid.*, **79**, 4563 (1957).

(3) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **76**, 3568 (1954).

(4) J. H. Boothe, S. Kushner, J. P. Petisi and J. H. Williams, *ibid.*, **75**, 3261 (1953).

(5) B. L. Hutchings, C. W. Waller, S. Gordon, R. W. Broschard, C. F. Wolf, A. A. Goldman and J. H. Williams, *ibid.*, **74**, 3710 (1952). For a discussion of and references to this type of tautomerism see ref. 4.

(6) Upon standing in 0.1 *N* sodium hydroxide for an hour or less the long wave length absorption maximum undergoes a hypsochromic shift which is reversible by acidification. This is assumed to be attributable to the opening and closing of the lactone ring and will be dealt with in more detail in a subsequent publication.

RESEARCH DIVISION — J. H. BOOTHE
ORGANIC CHEMICAL — A. GREEN
RESEARCH SECTION — J. P. PETISI
AMERICAN CYANAMID COMPANY — R. G. WILKINSON
PEARL RIVER, NEW YORK — C. W. WALLER

RECEIVED AUGUST 1, 1957

α,α′-DIGLYCEROPHOSPHATE IN PLANTS

Sir:

We have observed a P^{32}-labeled compound in hydrolysates of *Scendesmus* phosphatides which contained more than a third of the lipid phosphorus. The same phosphate ester also possessed as much as 90% of the alcohol-soluble non-lipid phosphorus of *Scenedesmus* cultured at low light intensity in media containing P^{32}. The cellular concentration of the ester, calculated from its P^{32} activity and the nutrient specific activity, was as high as 10^{-3} *M*. The same compound in lower concentrations occurred in the only two species of higher plants (clover) tested. It was isolated by chromatography on Whatman No. 1 paper with R_f = 0.36 in phenol–water and R_f = 0.11 in butanol–propionic acid–water.[1] These R_f values correspond to those recorded by Dawson[2] for an unknown in rat liver extracts.

(1) A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, THIS JOURNAL, **72**, 1710 (1950).

(2) R. M. C. Dawson, *Biochim. et Biophys. Acta*, **14**, 374 (1954).