Anal. Calcd. for $C_8H_{13}D$: C, 86.40; H, 13.60; mol. wt., 111. Found: C, 85.92; H, 14.47; mol. wt., 111 (by mass spectrometry[18]).

3-Deuteriocyclopentanone (XI).—The double bond isomerization was accomplished by heating on the steam-bath 2.6 g. of the olefin IX with 100 mg. of recrystallized naphthalenesulfonic acid. After 2 hr. the black solution was diluted with 20 cc. of methylene chloride and filtered through neutral alumina (activity II) to afford a light brown solution, whose infrared spectrum indicated the complete disappearance of the terminal olefin bands.

The methylene chloride solution was ozonized at −60° until a blue color persisted, whereupon nitrogen was bubbled through followed by shaking for 2.5 hr. with 20 cc. of 10% potassium hydroxide and 2 cc. of 33% hydrogen peroxide. The colorless organic phase was washed with water, dried over magnesium sulfate and concentrated to a small volume. Purification by gas phase chromatography using a Beckman Megachrom instrument and a Ucon Polar-Chromosorb column at 125° afforded 22 mg. of 3-deuteriocyclopentanone (XI), whose retention time was identical with that of cyclopentanone. The infrared spectrum exhibited a band (carbon tetrachloride) at 5.74 μ and analytical gas chromatography on a phenyldiethanolamine succinate column (100°) indicated a purity in excess of 90%. The mass spectrum[18] showed a strong molecular ion peak at mass 85 as well as the expected fragmentation peaks (by analogy to the mass spectrum[20] of cyclopentanone), such as m/e 56 ($C_4H_5D^-$) and m/e 42 ($C_3H_4D^+$), and indicated a minimum purity of 85% of 3-deuteriocyclopentanone (XI). The rotatory dispersion curve of the ketone was measured in isooctane solution (*c* 0.1) and showed no perceptible rotation in the ultraviolet (to 280 mμ) under conditions where with the identical concentration of androstan-17-one a rotation of $[\alpha]_{322}$ +42° would have been measurable.

(20) P. Natalis, *Bull. soc. chim. Belg.*, **67**, 599 (1959); J. H. Beynon, R. A. Saunders and A. E. Williams, *Appl. Spectros.*, **14**, 95 (1960).

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES, A DIVISION OF AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

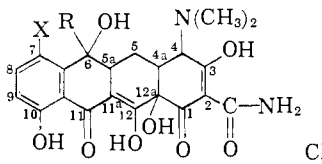
Total Synthesis of Tetracyclines. V. The Stereospecific Elaboration of the Tetracycline Ring System

BY THOMAS L. FIELDS, ANDREW S. KENDE AND JAMES H. BOOTHE

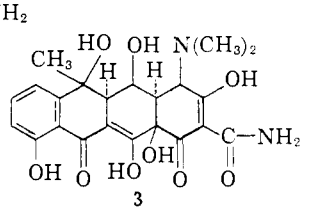
RECEIVED APRIL 28, 1961

The *syn*-(**10**) and *anti*-(**19**) isomers of 5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic acid have been synthesized from the bicyclic intermediate 3-(5-benzyloxy-8-chloro-1,2,3,4-tetrahydro-4-oxo-2-naphthylmethyl)-glutaric acid (**6**). Elaboration of the *syn*-acid **10** into (±)-7-chloro-dedimethylamino-6-demethyl-6,12a-dideoxytetracycline (**17**) and comparison with a sample of **17** obtained by degradation, are described.

One of the more formidable problems associated with the total synthesis of the tetracycline antibiotics arises from the number of asymmetric centers in the molecule.[1] Tetracycline (**1**) itself contains five asymmetric carbon atoms, those numbered 4, 4a, 5a, 6 and 12a. The asymmetric center at 4 is readily epimerized at intermediate *p*H ranges[2] and need not be of early concern in any synthetic scheme. With regard to the angular position C-12a, Holmlund, Andres and Shay[3] have shown that the insertion of the 12a-hydroxyl in the required configuration can be achieved for certain 12a-deoxytetracycline derivatives by gentle oxidative techniques. Furthermore, the catalytic hydrogenolysis of the C-6 hydroxyl group of the 6-demethyltetracyclines[4] does not appreciably affect their antibacterial activity.[5] Therefore, only the asymmetric carbon atoms 4a and 5a are of prime importance in a total synthetic approach to a biologically active tetracycline antibiotic. Since these centers, unlike positions 4 and 12a, are not subject to direct chemical manipulations when part of the intact tetracyclic molecule, their stereoselective formation must be a prerequisite of any successful synthetic scheme.



1, R = CH_3, X = H
2, R = H, X = Cl



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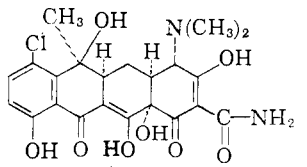


4

Although chemical evidence bearing on the stereochemistry of positions 4a and 5a is scanty, Woodward, *et al.*,[6] have suggested that the highly enolic character of 5-hydroxytetracycline (**3**) and its derivatives points toward a *syn* relationship.

(1) For a more complete discussion of the problems involved in the synthetic work see the previous paper in this series: A. S. Kende, T. L. Fields, J. H. Boothe and S. Kushner, *J. Am. Chem. Soc.*, **83**, 439 (1961).

(2) (a) A. P. Doerschuk, B. A. Bittler and J. R. D. McCormick, *ibid.*, **77**, 4687 (1955); (b) C. R. Stephens, L. H. Conover, P. N. Gordon, F. C. Pennington, R. L. Wagner, K. J. Brunings and F. J. Pilgrim, *ibid.*, **78**, 1515 (1956); (c) J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bittler, J. Reichenthal, V. E. Origoni, W. H. Muller, R. Winterbottom and A. P. Doerschuk, *ibid.*, **78**, 3547 (1956); (d) J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bittler, J. Reichenthal, V. E. Origoni, W. H. Muller, R. Winterbottom and A. P. Doerschuk, *ibid.*, **79**, 2849 (1957).

(3) C. E. Holmlund, W. W. Andres and A. J. Shay, *ibid.*, **81**, 4748 (1959).

(4) (a) J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen, A. P. Doerschuk, *ibid.*, **79**, 4561 (1957); (b) J. S. Webb, R. W. Broschard, D. B. Cosulich, W. J. Stein and C. F. Wolf, *ibid.*, **79**, 4563 (1957); (c) J. H. Boothe, A. Green, J. P. Petisi, R. G. Wilkinson and C. W. Waller, *ibid.*, **79**, 4564 (1957).

(5) J. R. D. McCormick, E. R. Jensen, P. A. Miller and A. P. Doerschuk, *ibid.*, **82**, 3381 (1960).

(6) 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).

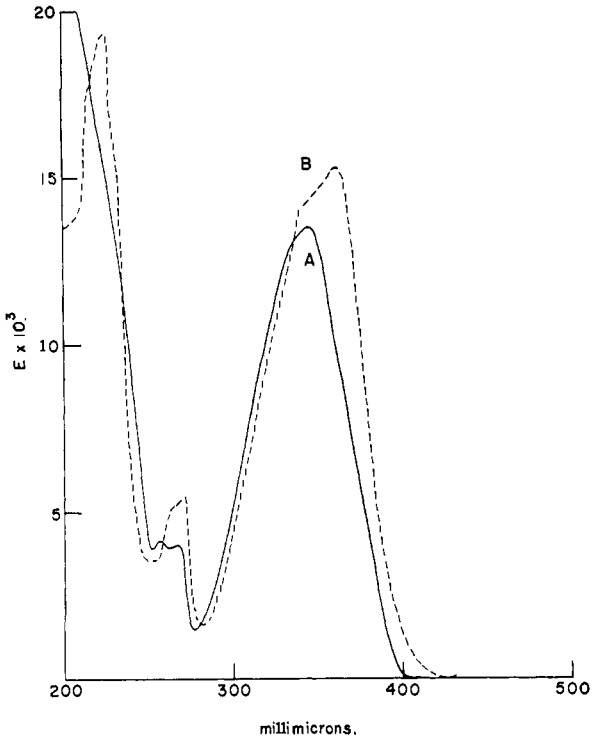


Fig. 1.—Ultraviolet absorption spectra of: A, *syn*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic acid (**10**) in methanol; B, *syn*-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-5-hydroxy-4,10-dioxo-2-anthraceneacetic acid (**12**) in methanol.

The recently completed X-ray diffraction analysis of 7-chlorotetracycline (**4**) hydrochloride has confirmed this assignment.[7] Our initial efforts toward the synthesis of tetracycline derivatives had involved the formation of a tricyclic system by a Claisen-type cyclization of the glutaric diester **5**. A single crystalline tricyclic product **9**, m.p. 118–121°, was isolated from this reaction and was assigned *syn* stereochemistry on conformational grounds.[1,8] Although the conversion of **9** to the anhydrotetracycline derivative **16** was subsequently achieved, attempts to utilize directly the stereochemically appropriate system of **9** for construction of the tetracycline derivative **17** were unsuccessful, since our proximate objective, the tricyclic *syn*-acid **10**, could not be obtained by direct alkaline hydrolysis of the corresponding ester **9**. Treatment of ester **9** with a variety of hydrolytic systems, including 1 *N* sodium hydroxide and 2 *N* sodium carbonate, led to cleavage of the β-dicarbonyl system at a rate comparable to saponification.

In view of these difficulties, it seemed advisable first to remove the benzyl group in the hope of stabilizing the labile β-dicarbonyl system. The free phenol **11** was readily obtained by hydrogenolysis of the benzyl group in methyl Cellosolve with 10% palladium-on-carbon as catalyst. The crude phenol **11** was subjected to alkaline hydrolysis, and the desired tricyclic acid **12** was isolated in 72% over-all yield. The increased alkali stability of the phenolic β-dicarbonyl system as compared to the blocked system may possibly be accounted for by the increased acidity presumably achieved by removal of the benzyl group.[9,10] The tricyclic acid **12** was converted to the acid chloride **13** by refluxing in benzene with oxalyl chloride.[11] The acyl malonate **14** was readily obtained by treatment of **13** with diethyl ethoxy magnesiomalonate. Attempts to cyclize **14** to the desired tetracyclic system with sodium hydride as the condensing agent proved unsuccessful.

Because of the above difficulties, it seemed desirable to devise a method for obtaining the benzyloxy acid **10** directly from a cyclization reaction. Accordingly, the substituted glutaric acid **6** was con-

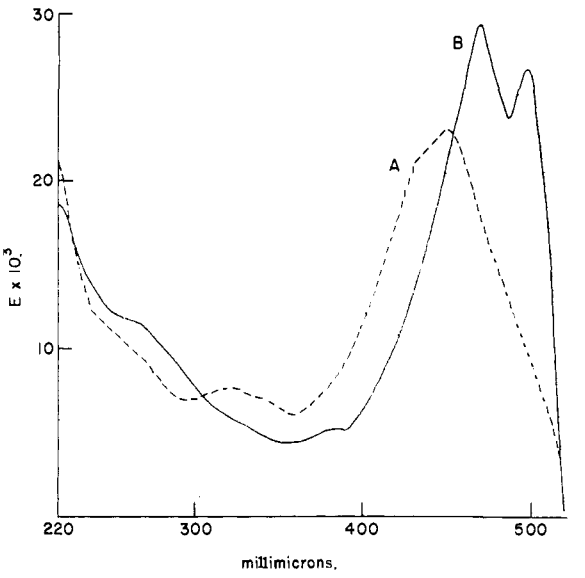
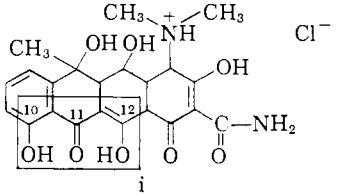


Fig. 2.—Ultraviolet absorption spectra of: A, ethyl 7-chloro-1,2,3,4,4a,5,5a,6,11,11a,12,12a-dodecahydro-10-hydroxy-1,3,11,12-tetraoxo-2-naphthacenecarboxylate (**30**); B, (±)7-chlorodedimethylamino-6-demethyl-6,12a-dideoxytetracycline (**17**) in 0.001 *N* methanolic sodium hydroxide.

(7) S. Hirokawa, Y. Okaya, F. M. Lovell and R. Pepinsky, Abstr. of Amer. Cryst. Assocn. Meeting, Cornell University, July, 1959, p. 44.

(8) J. H. Boothe, A. S. Kende, T. L. Fields and R. G. Wilkinson *J. Am. Chem. Soc.*, **81**, 1006 (1959).

(9) A thorough study of the acidity constants of the tetracyclines has been carried out by C. R. Stephens, K. Murai, K. J. Brunings and R. B. Woodward, *ibid.*, **78**, 4155 (1956), in which they show that the acidity of the C-10, C-11, C-12 grouping in 5-hydroxytetracycline hydrochloride (i) is reduced by 2.5 pK_a units when the phenol is blocked.

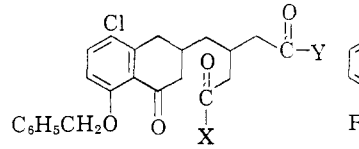


i

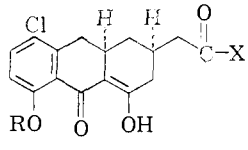
(10) The increased acidity of the phenolic dicarbonyl system would increase the concentration of resonance-stabilized enolate anion in alkaline solution, resulting in a diminished concentration of undissociated ketone available for attack by base (*cf.* E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, pp. 337, 376.

(11) The reactivity of the β-dicarbonyl system of the benzyloxy acid **10** as compared to the phenolic acid **12** is further illustrated by their behavior toward oxalyl chloride. As noted above, the hydroxy acid **12** gives the acid chloride in good yield, whereas the benzyloxy acid **10** gives an unidentified product having five distinct bands in the carbonyl region of its infrared spectrum.

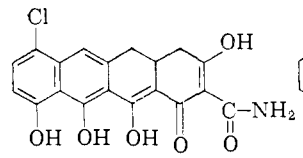
verted to the anhydride **18** by refluxing in acetic anhydride for one hour. Formation of the diastereomeric monoesters **7** and **8** was next achieved in good yield by the addition of one equivalent of sodium methoxide to a suspension of the anhydride in absolute methanol. All attempts to separate the mixture of esters (**7** and **8**) were unsuccessful. Therefore, the mixture itself was cyclized, using sodium hydride in refluxing toluene, and the tricyclic acids **10** and **19** were separated by crystallization from ethanol. It was observed that one of the isomers, m.p. 198–199°, was formed in the cyclization more rapidly and in higher yield (30–35%) than the other isomer, m.p. 177–179° (10–15%).



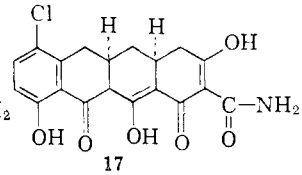
5, X = Y = OCH_3
6, X = Y = OH
7, X = OH, Y = OCH_3
8, X = OCH_3, Y = OH



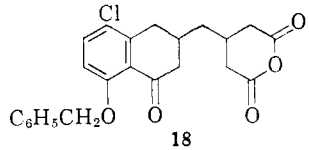
9, R = $CH_2C_6H_5$, X = OCH_3
10, R = $CH_2C_6H_5$, X = OH
11, R = H, X = OCH_3
12, R = H, X = OH
13, R = H, X = Cl
14, R = H, X = $CH(COOC_2H_5)_2$
15, R = $CH_2C_6H_5$, X = $CH(COOC_2H_5)_2$



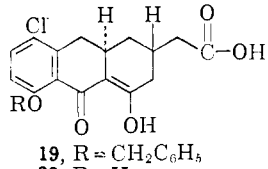
16



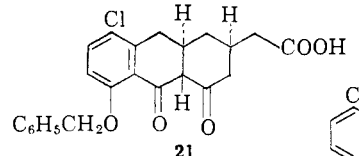
17



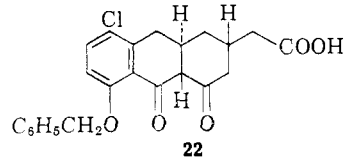
18



19, R = $CH_2C_6H_5$
20, R = H



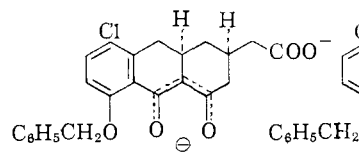
21



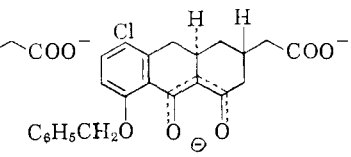
22

Although the exact geometries of the transition states leading to the products is uncertain, they probably resemble, to a considerable extent, the tricyclic diketones **21** and **22**.[12] A total of six

(12) Under the conditions of the reaction, the diketones **21** and **22** would undergo rapid and irreversible proton loss to give the corresponding enolate anions i and ii.

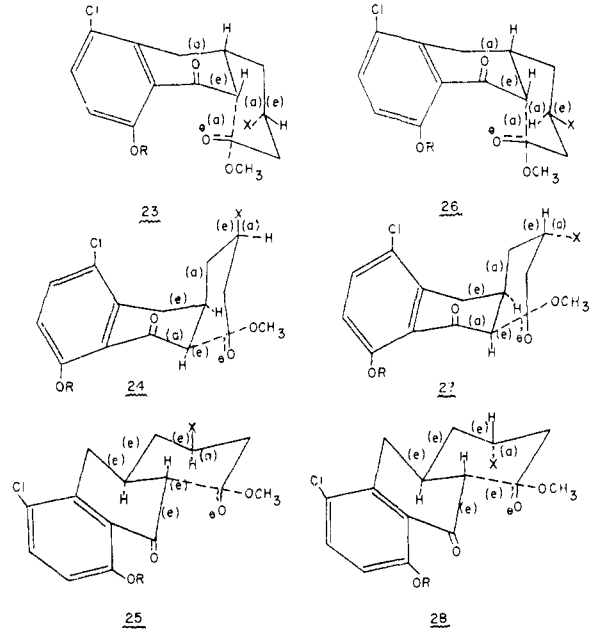


i



ii

chair conformations for the incipient cyclohexanone ring may be postulated. Of the three possible structures (**23** through **25**) leading to *syn* product **10**, that transition state (**25**) yielding the *trans-syn*-diketone **21** possesses the fewest unfavorable non-bonded interactions. Similarly, of the three possible structures leading to *anti* product **19**, that transition state (**28**) yielding *trans-anti*-diketone **22** is sterically favored. Since the most favorable chair conformation (**25**) leading to *syn*-acid **10** contains one less 1,3-diaxial carbon-hydrogen interaction and one less 1,3-diaxial carbon–oxygen interaction than the most favorable chair conformation (**28**) leading to *anti*-acid **19**. one would expect the *syn*-acid **10** to be formed more rapidly than the *anti*-acid **19**. On this basis the isomer, m.p. 198–199°, was assigned the *syn* stereochemistry.



23 thru 28: R = $CH_2C_6H_5$, X = CH_2COOH

23 through 28: R = $CH_2C_6H_5$, X = CH_2COOH

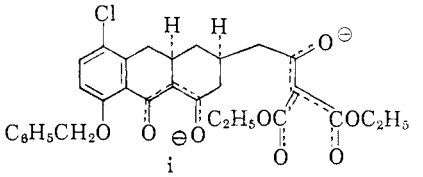
Structure	1,3-Diaxial interactions C-H	C-C	C-O
23 (*cis-syn*)	1	1	2
24 (*cis-syn*)	2	0	0
25 (*trans-syn*)	0	0	0
26 (*cis-anti*)	2	0	1
27 (*cis-anti*)	3	0	1
28 (*trans-anti*)	1	0	1

In addition, the major product (**10**) of this cyclization was related to the previously described[8] *syn*-ester **9** in the following manner. Hydrogenolysis of **10** over palladium-on-carbon gave a phenolic tricyclic acid, m.p. 204–205.5°, that was identical to the acid **12** obtained by hydrogenolysis and hydrolysis of the *syn*-ester **9**. Moreover, esterification of **10** with diazomethane gave a methyl ester, m.p. 120–122°, identical to the *syn*-ester **9** obtained by cyclization of the glutaric diester **5**. On the other hand, debenzylation of the minor product **19** gave the heretofore unreported *anti*-acid **20**, m.p. 170–172°.

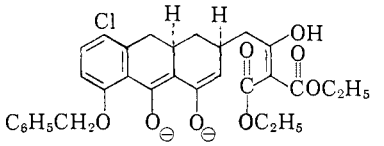
Having thus established the relative stereochemistry of the potential 4a,5a-carbons, we com-

menced the elaboration of *syn*-acid **10** to the polycarbonyl hydronaphthacene framework of the tetracyclines. The additional three carbon unit was readily incorporated by acylation of diethyl malonate with **10** *via* the mixed anhydride procedure. Cyclization of the resulting acylmalonate **15** in refluxing toluene, using sodium hydride as the condensing agent, gave a 30% yield of the desired *syn*-tetracyclic ester **29**.[13,14] This product was characterized by lack of carbonyl absorption in the infrared below 6.0 μ, absorption in the ultraviolet at 430 mμ in methanolic hydrochloric acid [15]

(13) The facile cyclization of the polycarbonyl compound **15** was somewhat surprising, since it seems to involve the addition of a highly unstable carbanion to an electron-rich center. Thus in the presence of sodium hydride one would expect the acylmalonate **15** to exist largely in the dianion form (i). We suggest that the cyclization could occur after an intramolecular proton transfer (i → ii).

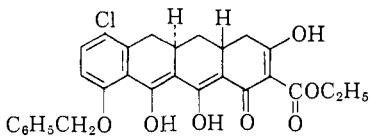


i

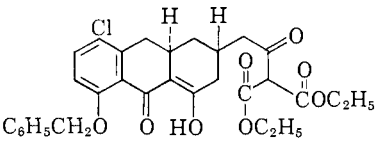


ii

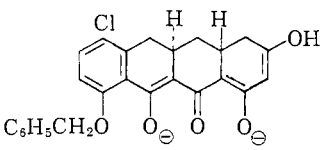
(14) Attempts to prepare the corresponding *anti* tetracyclic ester i by closure of ii were unsuccessful. An examination of Dreiding models suggests that substantial torsional strain is introduced when an attempt is made to hold the lower periphery (carbon atoms 10a, 11, 11a, 12, 12a, 1) of i fully planar, as would be optimal for formation of the cyclization product, the dianion iii.



i



ii

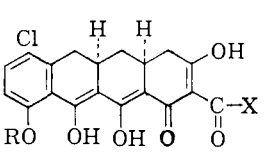


iii

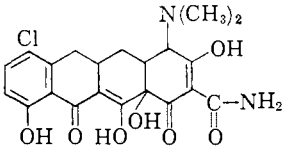
(15) The lack of carbonyl absorption below 6.0 μ is of significance in assigning structure **29** to the reaction product rather than structure i, which could result from cleavage of the initially formed ester **29** followed by recyclization to i. The analogous compounds ii, reported by R. G. Wilkinson, T. L. Fields and J. H. Boothe [*J. Org. Chem.*, **26**, 637 (1961)] and iii, reported by J. H. Boothe, A. S. Kende, T. L. Fields and R. G. Wilkinson (ref. 8), show no carbonyl absorption below 6.0 μ, whereas the 4-carbethoxy compound iv reported by H. Muxfeldt, W. Rogalski and K. Striegler [*Angew. Chem.*, **72**, 170 (1960)] exhibits a carbonyl peak at 5.82 μ (H. Muxfeldt, private communication). It is noteworthy that the tetraketone iv has $\lambda_{max}^{MeOH/HCl}$ 324 mμ, some 115 mμ below the related tetracyclic system of phenol **30**. Professor Muxfeldt has suggested (private communication) that the apparent failure of his compound iv to enolize at C-11a probably results from the unfavorable steric interaction between the C-6 methyl and the C-7 chlorine which would occur whenever C-11a is trigonal rather than tetrahedral.

and the generation of naphthacene on zinc dust pyrolysis.

Hydrogenolysis of **29** over palladium-on-carbon yielded the corresponding phenol **30.** Fusion of the latter with ammonium formate at 140° and subsequent strong acid hydrolysis gave a small yield of the desired racemic amide **17.**



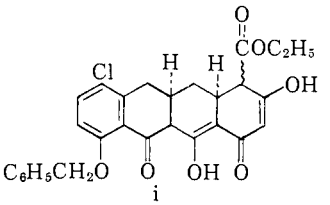
29, R = $CH_2C_6H_5$, X = OC_2H_5
30, R = H, X = OC_2H_5



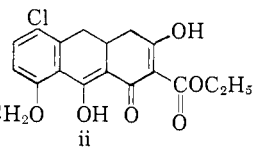
31

The preparation of optically active amide **17** by chemical transformations of a naturally occurring antibiotic was accomplished in the following manner. Natural 7-chloro-6-demethyltetracycline (**2**) was converted to the corresponding 6-deoxy derivative **31** by catalytic reduction using 30% rhodium-on-carbon as the catalyst.[16,17] Purification of this material was achieved by chromatography on Celite using a butanol–chloroform–*p*H 2 buffer system. Removal of the dimethylamino and 12a-hydroxyl groups was carried out with zinc and acetic acid.[18] The optically active amide **17** thus obtained was shown to be chemically identical to the totally synthetic amide **17** by paper chromatography, infrared and ultraviolet spectroscopy.

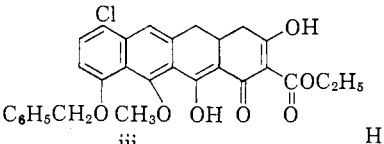
Acknowledgment.—We wish to thank Mr. W. Fulmor and his associates for spectroscopic determinations, Mr. L. Brancone and staff for microanalysis and Miss R. Livant for paper chromatographic studies. We are also indebted to Dr.



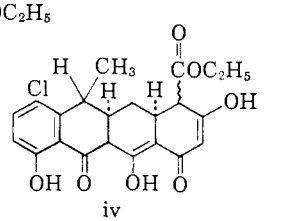
i



ii



iii



iv

(16) J. R. D. McCormick and E. R. Jensen, German Patent 1,082,905, June 9, 1960.

(17) We are indebted to J. R. D. McCormick and E. R. Jensen for the specific directions for the isolation and purification of this compound.

(18) 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, *J. Am. Chem. Soc.*, **74**, 4976 (1952); **76**, 4564 (1954).

H. Arlt and his staff for the large scale preparation of certain intermediates. We are indebted to Dr. R. G. Wilkinson for his contribution to the development of the basic approach used in this synthesis.

Experimental[19]

Attempted Hydrolysis of Methyl 5-Benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-anthracene-2-acetate (9).—Approximately 30 mg. of crude tricyclic diketo methyl ester **9** was refluxed under nitrogen in 20 ml. of 1 *N* sodium hydroxide. After 2 hours the ultraviolet maxima had shifted from 360 to 345 mμ in base, indicative of cleavage of the ring system. Similar results were obtained when the ester was heated on a steam-bath with 2 *N* sodium carbonate.

***syn*-8-Chloro-1,2,3,4,4a,9,9a,10-octahydro-5-hydroxy-4,10-dioxo-anthracene-2-acetic Acid (12).**—A solution of methyl *syn*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-anthracene-2-acetate (**9**) (854 mg., 0.002 mole) in 50 ml. of methyl Cellosolve containing 4 drops of glacial acetic acid and 100 mg. of 10% palladium-on-carbon was subjected to hydrogenation at room temperature. After 40 minutes, 1.2 equivalents of hydrogen had been taken up and the reaction was stopped. The catalyst was filtered off and the filtrate concentrated to an oil *in vacuo*. The crude debenzylated material was dissolved in 75 ml. of 1 *N* NaOH and refluxed under nitrogen for 1 hour. The hydrolyzed solution was cooled to room temperature and acidified with hydrochloric acid. The tan solid which precipitated was collected on a filter, washed with water and dried *in vacuo* over P_2O_5 at 60°. The yield of crude *syn*-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-5-hydroxy-4,10-dioxo-anthracene-2-acetic acid (**12**) was 468 mg. (72.5%). An analytical sample was obtained by recrystallization from methanol; bright yellow crystals, m.p. 206–208°; λ_{max}^{KBr} 5.85, 6.2, 6.34 and 6.89 μ; λ_{max}^{MeOH} 360 mμ log ε 4.18; λ_{max}^{NaOH} 375 mμ log ε 4.25.

Anal. Calcd. for $C_{16}H_{15}O_5Cl$: C, 59.53; H, 4.68; Cl, 10.98. Found: C, 59.42; H, 4.94; Cl, 11.17.

Attempted Preparation of Ethyl *syn*-7-Chloro-1,2,3,4,4a,5,5a,6,11,11a,12,12a-dodecahydro-10-hydroxy-1,3,11,12-tetraoxo-2-naphthacenecarboxylate (30).—A suspension of *syn*-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-5-hydroxy-4,10-dioxo-2-anthraceneacetic acid (64 mg.) in 7 ml. of benzene and 0.2 ml. of oxalyl chloride was refluxed for 45 minutes. The clear yellow solution was cooled and concentrated to dryness *in vacuo*. The oily residue was redissolved in benzene and again concentrated to dryness *in vacuo* in order to remove any residual oxalyl chloride. The infrared spectrum of the gummy residue exhibited a very strong maxima at 5.56 μ (acid chloride carbonyl) and a considerably weaker maximum at 5.86 μ, presumably due to unreacted acid. The crude acid chloride was dissolved in 5 ml. of sodium-dried toluene and added dropwise to a solution of diethyl magnesio-malonate (0.0006 mole) in 10 ml. of toluene. A fine white solid precipitated after a few minutes. The suspension was stirred at room temperature overnight. The reaction was acidified by the addition of 10 ml. of 1 *N* hydrochloric acid. The organic layer was separated, washed twice with 10 ml. of water, dried over anhydrous magnesium sulfate, filtered and concentrated to an oil *in vacuo*. Unreacted diethyl malonate was removed by heating at 90° at 1 mm. for 5 hours. The crude acyl malonate was dissolved in 10 ml. of sodium-dried toluene and an excess of sodium hydride in oil added. The mixture was refluxed in a nitrogen atmosphere and aliquots taken at 4 and 20 hours. The ultraviolet spectra of the aliquots were identical to the spectra of the starting acyl malonate (λ_{max}^{MeOH/H^+} – ~360 mμ). After 24 hours the reaction was cooled and the excess hydride decomposed by the careful addition of a few drops of absolute methanol and acidified with 10 ml. of 1 *N* HCl. Sufficient ethyl acetate was added to give two clear layers. The organic layer was separated, washed thrice with water, dried over anhydrous magnesium sulfate, filtered and concentrated to a gum *in vacuo*. The infrared spectrum of this material was quite similar to the starting acyl malonate. Zinc dust distillation of this material did not yield any naphthacene.

3-(5-Benzyloxy-8-chloro-1,2,3,4-tetrahydro-4-oxo-2-naphthylmethyl) glutaric anhydride (18). 3-(5-Benzyloxy-8-chloro-1,2,3,4-tetrahydro-4-oxo-2-naphthylmethyl)-glutaric acid (**6**) (900 mg., 0.00208 mole) was slurried in 15 ml. of acetic anhydride and refluxed for 1 hour. The clear solution was concentrated by distillation to approximately 5 ml. Upon cooling, the white crystals which deposited were collected on a filter, washed well with ether and dried *in vacuo* at 60°. The yield of pure anhydride was 743 mg., m.p. 192–193°.

Anal. Calcd. for $C_{23}H_{21}ClO_5$: C, 66.90; H, 5.13; Cl, 8.59. Found: C, 66.64; H, 5.32; Cl, 8.60.

Methyl 3-(5-Benzyloxy-8-chloro-1,2,3,4-tetrahydro-4-oxo-2-naphthylmethyl)-glutarate (7 and 8).—3-(5-Benzyloxy-8-chloro-1,2,3,4-tetrahydro-4-oxo-2-naphthylmethyl)-glutaric anhydride (**18**) (2.48 g., 0.006 mole) was added to a solution of sodium methoxide (0.0066 mole) in 60 ml. of absolute methanol and stirred at room temperature for 2 hours. The clear solution was diluted with 150 ml. of water and acidified by the addition of 10 ml. of 1 *N* hydrochloric acid. The oil which separated was extracted into ethyl acetate, washed with water and dried over anhydrous magnesium sulfate. The clear solution was concentrated to an oil *in vacuo*. Trituration of the oil with ether yielded 1.88 g. of the diastereomeric methyl esters, m.p. 113–127°. This material was used without further purification for the cyclization step.

Anal. Calcd. for $C_{24}H_{23}ClO_5$: C, 64.75; H, 5.66; Cl, 7.99. Found: C, 64.60; H, 5.91; Cl, 7.48.

5-Benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic Acid.—A suspension of monomethyl 3-(5-benzyloxy-8-chloro-1,2,3,4-tetrahydro-4-oxo-2-naphthylmethyl)-glutarate (4.5 g., 0.0101 mole) and sodium hydride in oil (2.5 g., 0.050 mole) in 300 ml. of sodium-dried toluene was refluxed in a nitrogen atmosphere for 48 hours. The yellow suspension was cooled to room temperature and the excess sodium hydride decomposed by the careful addition of glacial acetic acid. The reaction mixture was then diluted with 100 ml. of ethyl acetate and the clear solution washed thoroughly with 100 ml. of cold 1 *N* sulfuric acid. The organic layer was separated, washed thrice with 75-ml. portions of water, and dried over anhydrous magnesium sulfate. The solution was concentrated *in vacuo* and the residual gum was slurried in 30 ml. of ether. The yellow crystals which formed were collected on a filter, washed with ether and air-dried. The yield of crude tricyclic acid was 2.2 g. (53%), m.p. 180–190°. Two recrystallizations from absolute ethanol yielded 1.2 g. of pure *syn*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic acid (**10**), m.p. 198–199°. The mother liquor from the first recrystallization, upon chilling, yielded 65 mg. of pure *anti*-acid **19**, m.p. 178–180°.

The mother liquor from the crude tricyclic acid was concentrated to a gum *in vacuo*. The ultraviolet and infrared spectra of this material indicated that it was mainly bicyclic. This material was then dissolved in 150 ml. of sodium-dried toluene, sodium hydride in oil (1.2 g., 52%) was added and it was refluxed under nitrogen for 96 hours. The reaction mixture was then worked up as above to yield 0.5 g. of crude tricyclic acid, m.p. 165–170°. Attempts to obtain pure *syn*-acid **10** from this material were unsuccessful. However, crystallization from ethanol yielded 229 mg. of pure *anti*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic acid (**19**), m.p. 177–179°. An additional 262 mg. of tricyclic acid was obtained from the mother liquor whose m.p. (169–178°) and infrared spectra indicated that it was predominantly *anti* isomer.

Anal. Calcd. for $C_{23}H_{21}ClO_5$: C, 66.90; H, 5.13. Found: (*syn*) C, 66.57; H, 5.54; (*anti*) C, 66.58; H, 5.41.

Esterification of syn-5-Benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic Acid (10).—Diazomethane (20 ml. of a 1% ether solution) was added to a solution of *syn*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic acid (**10**), (33 mg. in 1.5 ml. of tetrahydrofuran). The solution was allowed to stand at 5–10° for 60 seconds, with frequent shaking. Five drops of acetic acid was added to destroy the excess diazomethane and the solution was quickly warmed to drive off any residual diazomethane. Evaporation of the solvent gave a gum which was taken up in 0.5 ml. of ether. Ethanol was added dropwise and the solution cooled and scratched vigorously. Crystals of the crude methyl ester were obtained, m.p. 110–114°. Recrystallization from *ca.* 0.4 ml. of 2:1 ethanol–

(19) All melting points were taken in soft glass capillaries and are uncorrected.

ether failed to raise the melting point. The crude material was taken up in ether and washed with 2% aqueous sodium bicarbonate solution, then with water. The ether solution was dried over anhydrous magnesium sulfate and then evaporated to dryness. Crystallization from ethanol–ether gave essentially pure methyl *syn*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetate (**9**) m.p. 116–118° (Kofler). The mixed melting point with *syn* ester **9** obtained by cyclization of methyl 3-(5-benzyloxy-8-chloro-1,2,3,4-tetrahydro-4-oxo-2-naphthylmethyl)glutarate (**5**) was 117–121°. The infrared spectra of the diazomethane product and the cyclization product were identical.

Esterification of *anti*-5-Benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic Acid (19).—A solution of *anti*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic acid (**19**) (160 mg.) in 2 ml. of tetrahydrofuran was mixed thoroughly with 20 ml. of a cold 1% solution of diazomethane in ether. After 90 seconds the excess diazomethane was destroyed with acetic acid. Evaporation of the solvent gave a gum which on trituration with *ca.* 1 ml. of 2:1 ethanol–ether yielded 59 mg. of crude *anti* ester, m.p. 95–97° (Kofler).

43 mg. of the crude ester was dissolved in ether–benzene and washed twice with 2% sodium bicarbonate solution and once with water. The organic layer was dried over anhydrous magnesium sulfate and then concentrated to an oil. Trituration with 0.3 ml. of ethanol–ether (2:1) gave 10 mg. of pure methyl *anti*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetate, m.p. 105–107°. The mixed melting point of this product with the isomeric *syn* ester was 98–121°. The infrared spectra of the two compounds differed at several points.

Anal. Calcd. for $C_{23}H_{23}O_5Cl$: C, 66.58; H, 5.59. Found: C, 66.23; H, 5.84.

***syn*-8-Chloro-1,2,3,4,4a,9,9a,10-octahydro-5-hydroxy-4,10-dioxo-2-anthraceneacetic Acid (12).**—A solution of *syn*-5-benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-5-hydroxy-4,10-dioxo-2-anthraceneacetic acid (**10**) (35 mg., 0.000108 mole) in 10 ml. of methyl Cellosolve containing 1 drop of acetic acid and 10 mg. of 10% palladium on carbon was subjected to hydrogenation at room temperature. After 40 minutes 1.03 equivalents of hydrogen had been taken up and the reaction was stopped. The catalyst was filtered off and the filtrate concentrated *in vacuo* to a yellow solid. The crude acid (**12**) was recrystallized from 3 ml. of methanol to yield 15 mg. of pale yellow crystals, m.p. 204–205.5° (54.5%). A second recrystallization from methanol raised the m.p. to 206–208°. The melting point was not depressed when admixed with the material obtained by hydrogenolysis and saponification of the *syn*-tricyclic benzyloxy ester **9**. The infrared spectra of the two samples were identical.

***anti*-8-Chloro-1,2,3,4,4a,9,9a,10-octahydro-5-hydroxy-4,10-dioxoanthracene-2-acetic acid (20).**—*anti*-5-Benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxoanthracene-2-acetic acid (50 mg.) was slurried in 1.5 ml. of glacial acetic acid. An equal volume of concentrated hydrochloric acid was added and the reaction mixture was refluxed under nitrogen for one hour. Upon cooling to room temperature pale yellow crystals precipitated. This material was collected on a filter, washed well with water and dried at 100° over phosphorus pentoxide *in vacuo*. The yield of debenzylated acid was 24 mg., m.p. 170–172°.

Anal. Calcd. for $C_{16}H_{15}O_5Cl$: C, 59.53; H, 4.68. Found: C, 59.12; H, 4.94.

Ethyl 10-Benzyloxy-7-chloro-1,2,3,4,4a,5,5a,6,11,11a,12,12a-dodecahydro-1,3,11,12-tetraoxonaphthacene-2-carboxylate (29).—5-Benzyloxy-8-chloro-1,2,3,4,4a,9,9a,10-octahydro-4,10-dioxo-2-anthraceneacetic acid (*syn* isomer **10**) (600 mg., 0.00145 mole) was slurried in 60 ml. of sodium dried toluene contained in a 125-ml. round-bottom flask protected with a calcium chloride tube. Triethylamine (0.48 ml., 0.00348 mole) was added and the cloudy suspension cooled in an ice–methanol bath to −10°. Ethyl chloroformate (0.336 ml., 0.00348 mole) was added and the mixture stirred at −10° for 12 minutes. Diethyl magnesiomalonate (0.00348 mole in 8 ml. of toluene) was added, the ice-bath removed, and the mixture stirred at room temperature overnight. The reaction mixture was diluted with 45 ml. of ethyl acetate and 50 ml. of cold 1 *N* sulfuric acid. The organic layer was separated and washed successively with water, 1 *N* sulfuric acid, and water. The organic phase was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to a gum. Sodium hydride in oil (600 mg.) was added to a solution of the crude acylmalonate in 120 ml. of sodium dried toluene and the mixture refluxed in a nitrogen atmosphere.

After 20 hours, the bright orange-red suspension was cooled in an ice-bath and the excess sodium hydride decomposed by the cautious addition of glacial acetic acid. The reaction mixture then was diluted with 6 ml. of absolute ethanol and 120 ml. of ethyl acetate. The orange solution was washed with 1 *N* sulfuric acid and four times with water. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residual gum was dissolved in ether and upon chilling orange crystals deposited. Yield of tetracyclic ester **29** was 233 mg. (31.6%). A 50-mg. sample was recrystallized from 2 ml. of ethyl acetate. The yield was 34 mg., m.p. 151–154°, $\lambda_{max}^{0.1\ M\ Na_2B_4O_7}$ 440, 340, 265 mμ, log ε 4.20, 3.98, 4.05.

Anal. Calcd. for $C_{28}H_{25}ClO_7$: C, 66.08; H, 4.95. Found: C, 66.01; H, 5.54.

Ethyl 7-Chloro-1,2,3,4,4a,5,5a,6,11,11a,12,12a-dodecahydro-10-hydroxy-1,3,11,12-tetraoxo-2-naphthacenecarboxylate (30).—A solution of ethyl 10-benzyloxy-7-chloro-1,2,3,4,4a,5,5a,6,11,11a,12,12a-dodecahydro-1,3,11,12-tetraoxo-2-naphthacenecarboxylate (**29**) (100 mg., 0.000196 mole) in 140 ml. of methyl cellosolve containing 3 drops of glacial acetic acid and 20 mg. of 10% palladium on carbon was subjected to hydrogenation at room temperature. After 40 minutes 1.2 equivalents of hydrogen had been taken up and the reaction was stopped. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to yield 69 mg. (92%) of the phenolic tetracyclic ester **30**, m.p. dec. 162–164°, $\lambda_{max}^{0.1\ M\ Na_2B_4O_7}$ 450, 270 mμ, log ε 4.39, 4.05.

(±)-Dedimethylamino-6-demethyl-6,12a-dideoxychlorotetracycline (17).—Ethyl 7-chloro-1,2,3,4,4a,5,5a,6,11,11a,12,12a-dodecahydro-10-hydroxy-1,3,11,12-tetraoxo-2-naphthacenecarboxylate (**30**) (49 mg.) and approximately 2 g. of ammonium formate were mixed intimately in a small test-tube. The test-tube was partially immersed in a round-bottom flask containing xylene, and the xylene bath heated to reflux under a nitrogen atmosphere for one hour. During the heating, the organic portion gradually rose to the top of the molten formate as a dark-brown, hard layer. The reaction mixture was allowed to cool and the contents of the tube poured into a mixture of 100 ml. of ethyl acetate and water (1:1). Upon vigorous shaking, the organic material formed a clear amber solution with the ethyl acetate. The aqueous phase was removed and the amber solution washed with water, 1 *N* sulfuric acid and again with water. The ethyl acetate layer was dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* to yield 25 mg. of a brick-red crystalline solid.

The crude amidation product was hydrolyzed directly by adding 4 ml. of glacial acetic acid, 4 ml. of concentrated hydrochloric acid and 6 drops of water. The clear solution was warmed on a steam-bath and an amorphous solid began precipitating after 15 minutes. The reaction mixture was cooled and diluted with 100 ml. of water after 35 minutes. The suspension was extracted with three 50-ml. portions of ethyl acetate and the combined extracts washed with 1 *N* sulfuric acid and then with water. The ethyl acetate solution was dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The residual gum was slurried in 10 ml. of ether and the amorphous solid collected on a filter. Paper strip chromatography indicated that this material contained a large percentage of the desired tetracycline derivative **17**. The mother liquor from this material deposited 1 mg. of microcrystalline material upon chilling. The infrared and ultraviolet spectrum and paper chromatogram of this material were identical with those of optically active dedimethylamino-6-demethyl-6,12a-dideoxy-chlorotetracycline (**17**) obtained by degradation of the natural antibiotic 6-demethylchlorotetracline. $\lambda_{max}^{0.001\ N\ NaOH(MeOH)}$ 495, 469, 375, 262 mμ.

6-Deoxy-6-demethylchlorotetracycline (31).—6-Demethylchlorotetracycline (10.0 g.) was slurried in 240 ml. of dimethylformamide. Concentrated hydrochloric acid (3.5 ml.) was added and the resultant hazy solution was filtered. Ten grams of 30% rhodium on carbon were added and the mixture placed on a Parr shaker and contacted with an

excess of hydrogen at 50 lb. per square inch for four hours. The crude reaction mixture was filtered through Celite to remove the majority of the catalyst. The filtrate was concentrated *in vacuo* and the sirupy residue taken up in 200 ml. of distilled water and freeze-dried.

The crude reaction mixture was filtered through Celite to remove the majority of the catalyst. The filtrate was concentrated *in vacuo* and the sirupy residue taken up in 200 ml. of distilled water and freeze-dried.

The freeze-dried material from five such runs was combined and dissolved in 450 ml. of butanol saturated with water. The *p*H was adjusted to 2.0 and the solution filtered. The filtrate was chromatographed on a 6-inch column packed with 3.4 kg. of acid-washed Celite using a 80% BuOH–20% $CHCl_3$–*p*H 2.0 developer. A total of twelve 250-ml. cuts were taken. The composition of the cuts was determined by paper chromatography using a butanol–phosphate–*p*H 2.0 system.

Cuts 1 and 2 were essentially pure 6-deoxy-6-demethylchlorotetracycline. Cuts 3 through 9 were a mixture of 6-deoxy-6-demethylchlorotetracycline, 6-demethyl-6-deoxytetracycline and a small amount of starting material. Cuts 3 through 9 were combined and concentrated to 400 ml. The *p*H was adjusted to 2.0 and the solution filtered. The clear filtrate was columned as before. Paper strips indicated that cuts 1 through 7 were essentially pure 6-deoxy-6-demethylchlorotetracycline.

Cuts 1 and 2 from the first column and cuts 1 through 7 from the second column were concentrated to dryness *in vacuo*. The residue was taken up in 300 ml. of water the *p*H adjusted to 2.0 with hydrochloric acid and filtered. The filtrate was freeze-dried to yield 4.85 g. of 6-deoxy-6-demethylchlorotetracycline hydrochloride.

Further purification was accomplished as follows: 2.3 g. of the freeze-dried material was dissolved in 55 ml. of methanol and the hazy solution filtered. The *p*H of the clear filtrate was adjusted to 9.0 by the addition of triethylamine and then brought down to *p*H 1.5 by the addition of concentrated sulfuric acid. Upon stirring and scratching, bright yellow needles deposited. The yield of 6-deoxy-6-demethylchlorotetracycline (**31**) was 1.8 g.

Anal. Calcd. for $C_{21}H_{21}O_7ClN_2 \cdot H_2SO_4 \cdot H_2O$: C, 44.6; H, 4.46; N, 4.96; Cl, 6.28. Found: C, 44.60; H, 4.45; N, 4.75; Cl, 6.51.

Dedimethylamino-6-demethyl-6,12a-dideoxychlorotetracycline (17).—Zinc dust (500 mg.) was added to a solution of 6-deoxy-6-demethylchlorotetracycline (400 mg.) and anhydrous sodium acetate (65.5 mg.) in glacial acetic acid (20 ml.) and water (9 ml.). The mixture was stirred under nitrogen for 1.5 hours and an additional 500 mg. of zinc dust was added. After 6 hours the reaction mixture was diluted with 300 ml. of ethyl acetate, water (2:1) and the undissolved zinc filtered off. The aqueous layer was separated and extracted thrice with ethyl acetate. The combined ethyl acetate layers were washed with 1 *N* sulfuric acid and thrice with water. The organic layer was separated, dried over anhydrous magnesium sulfate and concentrated to dryness *in vacuo*. The residual orange solid was slurried in 10 ml. of toluene and again concentrated to dryness *in vacuo*. The yield of crude dedimethylamino-6-demethyl-6,12a-dideoxychlorotetracycline (**17**) was 97 mg. An analytical sample was obtained by washing with a small volume of chloroform. The infrared spectrum, the ultraviolet spectrum ($\lambda_{max}^{0.00\ N\ NaOH(MeOH)}$ 495, 469, 375, 262 mμ) and the chromatographic behavior (R_f 0.61 in a butyl acetate–formamide–acetone system using paper buffered at *p*H 6.0) of this material were identical to the corresponding properties of the synthetic amide **17**.

Anal. Calcd. for $C_{19}H_{16}NClO_6$: C, 58.6; H, 4.14; N, 3.60. Found: C, 58.69; H, 4.64; N, 3.88.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FORDHAM UNIVERSITY, NEW YORK 58, N. Y.]

Ozonolysis of Polycyclic Aromatics. VIII.[1] Benzo[a]pyrene[2,3]

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Ozonization of benzo[a]pyrene (I) in methylene chloride and 3:1 methylene chloride–methanol with one molar equivalent of ozone produced a mixture of quinones from which could be isolated benzo[a]pyrene-3,6-dione (II) and benzo[a]pyrene-1,6-dione (III) in 1:3 ratio, and trace amounts of benzo[a]-pyrene-4,5-dione. No solvent effects were observed. Ozonolysis of I with two molar equivalents of ozone gave 7H-benz[de]anthracen-7-one-3,4-dicarboxylic acid (IV) while four, six and eight molar equivalents of ozone produced only 1,2-anthraquinonedicarboxylic acid (VII). In all cases unreacted I was recovered. Ozonolysis of the quinone mixture also produced IV and VII. The stoichiometry of these various ozonizations is unclear but does suggest predominant attack at positions 1, 3 and 6 in I by more than one molecule of ozone. Several mechanisms are proposed. There now does not seem to be any simple correlation between K- and L- region additivity toward ozone and carcinogenicity in the series anthracene, benz[a]anthracene, dibenz[a,h]anthracene and I.

Introduction

Since our original investigation of the ozonolysis of phenanthrene,[4a,4b] we have actively investigated the reaction between ozone and polycyclic aromatic hydrocarbons. Our purpose was twofold: (i) to elucidate the nature of ozone attack on aromatic systems; and (ii) to correlate the relative carcinogenicity of these polycyclics with the course and mechanism of the ozonolysis reaction. Published results of our studies on naphthacene,[4c] benz[a]anthracene[4d,4e] and dibenz[a,h]anthracene[1,4f] have led us to note a simple correlation between carcinogenicity and K- and L-region activity toward ozone in this limited series.[4c,4f] In this paper we report on the ozonization of the strong carcinogen (++++)[5] benzo[a]pyrene (I).[6]

Results

Ozonization of I in methylene chloride, and methylene chloride–methanol (3:1) with one molar equivalent of ozone (3.5 vol. %) at −70°, and at 20°, gave a mixture of benzo[a]pyrene-3,6-dione (II), benzo[a]pyrene-1,6-dione (III) and benzo[a]-

(1) Part VII, E. J. Moriconi, W. F. O'Connor, W. J. Schmitt, G. W. Cogswell and B. P. Fürer, *J. Am. Chem. Soc.*, **82**, 3441 (1960).

(2) Presented in part at the Symposium on Ozone Chemistry, 135th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1959.

(3) This research was supported by a grant C-3325(C3) from the U. S. Public Health Service, National Cancer Institute.

(4) (a) W. J. Schmitt, E. J. Moriconi and W. F. O'Connor, *J. Am. Chem. Soc.*, **77**, 5640 (1955); (b) W. F. O'Connor, W. J. Schmitt and E. J. Moriconi, *Ind. Eng. Chem.*, **49**, 1701 (1957); (c) E. J. Moriconi, W. F. O'Conuor and L. B. Taranko, *Arch. Biochem. and Biophys.*, **83**, 283 (1959); (d) E. J. Moriconi, W. F. O'Connor and F. T. Wallenberger, *Chemistry and Industry*, 22 (1959); (e) E. J. Moriconi, W. F. O'Connor and F. T. Wallenberger, *J. Am. Chem. Soc.*, **81**, 6466 (1959); (f) E. J. Moriconi, G. W. Cogswell, W. J. Schmitt and W. F. O'Connor, *Chemistry & Industry*, 1591 (1958).

(5) G. M. Badger, *Brit. J. Cancer*, **2**, 309 (1948).

(6) L. F. Fieser and F. C. Novello [*J. Am. Chem. Soc.*, **62**, 1855 (1940)] have previously ozonized I "but the material appeared either to remain unchanged or to be attacked extensively, and no satisfactory products were isolated."