The compound was not active in the first three tumor systems. Table I lists the antitumor testing data against Lewis lung carcinoma, supplied by the CCNSC.

TABLE I
ANTITUMOR ACTIVITY OF 4-HYDROXY-2-BUTANONE THIOSEMICARBAZONE AGAINST LEWIS LUNG CARCINOMA

Dose, mg/kg	Survivors[a]	Av weight change, g, T/C	Av tumor wt, mg, T/C	T/C, %
400	5/6	−2.3	429/943	45
400	4/6	−3.1	478/1398	34
400	3/6	−1.5	648/811	79
400	4/6	−2.0	613/1404	43
400	3/6	−3.0	620/1010	61
400	5/6	−4.2	982/1957	50
400	5/6	−2.2	480/1086	44
400	4/6	−1.4	954/1440	66

[a] BDF1 mice

The 6-Deoxytetracyclines. VIII. Acylaminomethylamides

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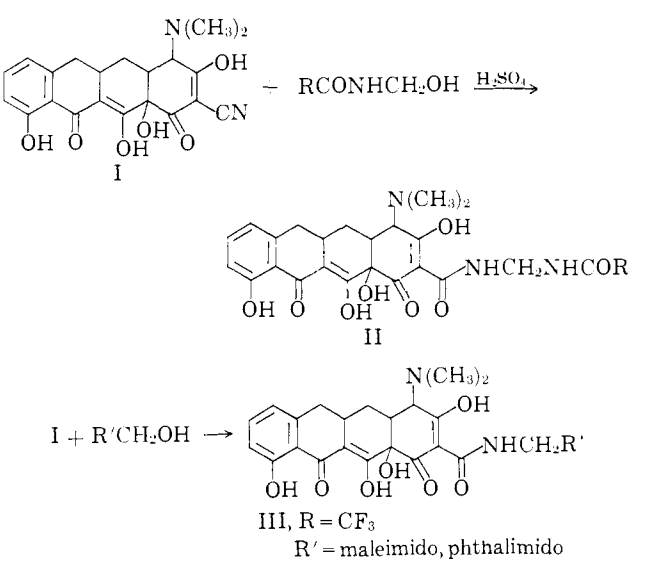
Although the facile conversion of the 2-carboxamido group in the tetracycline series into a nitrile by means of an acid chloride, such as benzenesulfonyl or methanesulfonyl chloride in pyridine has been known for some time,[1] only one reaction has appeared which utilizes the nitrile.[2] In that case the Ritter[3] reaction proceeded in a concentrated sulfuric acid–acetic acid mixture on 7-chlorotetracycline nitrile itself with isobutylene giving as products 2-carboxamido-N-*t*-butylanhydrochlortetracycline and the 9-*t*-butyl-*t*-butyl anhydroamide. These compounds have been recently photooxidized[4] by the method of Scott and Bedford.[5]

We now wish to report the reaction of 2-decarboxamido-2-cyano-6-deoxy-6-demethyltetracycline (I) with N-hydroxymethylimides or N-hydroxymethylamides to give acylaminomethylamides (II and III) (Scheme I).

The reaction of nitriles with N-hydroxymethylphthalimide in concentrated H_2SO_4 was reported in 1947 by Buc[6] predating that of the Ritter reaction.[3] The stabilized carbonium ion species involved is well known and its reaction with aromatic nuclei (Tscherniac–Einhorn reaction) has been recently excellently reviewed by Zaugg and Martin[7] as well as by others.[8]

SCHEME I

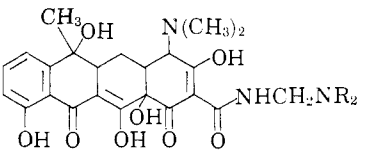


The reactions of these substances with the aromatic ring of tetracyclines are reported by us in an accompanying paper.

$$RCONH\overset{+}{C}H_2 \longleftrightarrow RCO\overset{+}{N}H{=}CH_2$$

Thus, 2-decarboxamido-2-cyano-6-demethyl-6-deoxytetracycline (I), when treated with 1 equiv of N-hydroxymethylphthalimide, N-hydroxymethyltrifluoroacetamide,[9] or N-hydroxymethylmaleimide[10] in concentrated H_2SO_4, gave the corresponding substituted amides in good yield which were readily purified by liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth.

The nitriles such as I in the tetracycline series are extremely resistant to hydrolysis, and extensive epimerization at 4 and decomposition usually accompany it.[11] The *t*-butyl-substituted anhydroamides previously alluded to have been hydrolyzed to the unsubstituted amide by strong acid treatment.[2c] However, the acylaminomethylamides described above can be hydrolyzed much more easily than the nitriles from which they are made. They are not, however, as easily decomposed as are the "Mannich" tetracyclines[12] IV which are readily hydrolyzed by even dilute acids. These derivatives are easily formed from tetracycline, formaldehyde, and a dialkylamine.



IV, R = alkyl

(1) 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, *J. Am. Chem. Soc.*, **75**, 5455 (1953); 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); J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Bitler, J. Reichenthal, V. E. Origoni, W. M. Muller, R. Winterbottom, and A. P. Doerschuk, *ibid.*, **79**, 2849 (1957).

(2)(a) 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); (b) C. R. Stephens, U. S. Patent 3,028,409 (April 3, 1962); (c) P. N. Gordon, U. S. Patent, 3,029,284 (April 10, 1962).

(3) J. J. Ritter and P. Minieri, *J. Am. Chem. Soc.*, **70**, 4045, 4048 (1948).

(4) M. Schach von Wittenau, *J. Org. Chem.*, **29**, 2746 (1964).

(5) A. I. Scott and C. T. Bedford, *J. Am. Chem. Soc.*, **84**, 2271 (1962).

(6) S. R. Buc, *ibid.*, **69**, 254 (1947).

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(8) R. Schröter in Houben-Weyl "Methoden der Organischen Chemie," Vol. XI/1, 4th ed, G. Thieme, Stuttgart, 1957, pp 795–805; 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.

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(11) J. J. Beereboom and K. Butler, U. S. Patent, 3,069,467 (Dec 18, 1962).

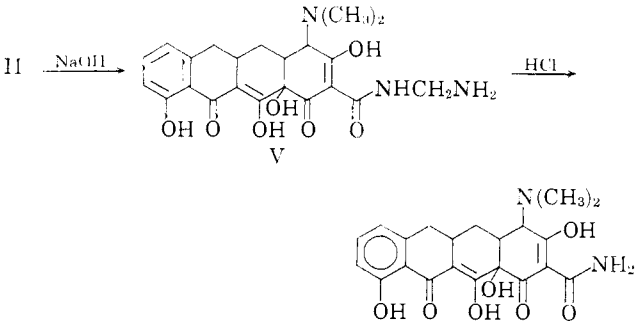
(12) W. Seidel, A. Soder, and F. Lindner, *Muench. Med. Wochschr.*, **17**, 661 (1958); W. J. Gottstein, W. F. Minor, and L. C. Cheney, *J. Am. Chem. Soc.*, **81**, 1198 (1959).

TABLE I

Derivative of 6-deoxytetracycline	R_f[a]	Partition chromatography data[b]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
2-Carboxamido-N-phthalimidomethyl-6-demethyl-	0.83	C–D–W (5:5:1) HBV[c] 2.0–3.0	$C_{30}H_{25}N_3O_9 \cdot 0.5C_4H_{10}O_2$[d]	62.25	5.106	6.80	62.51	5.27	6.42
2-Carboxamido-N-maleimidomethyl-6-demethyl-	0.69	H–EA–M–W (40:60:17:6) HBV 2.7–3.6	$C_{28}H_{25}N_3O_9 \cdot 0.5H_2O$	58.64	4.92	7.89	58.30	5.106	7.52
2-Carboxamido-N-trifluoroacetamidomethyl-6-demethyl-	0.88	H–EA–ME–W (35:65:17:6) HBV 2.0–3.0	$C_{24}H_{24}F_3N_3O_8$[e]			7.79			7.95

[a] 1-Butanol–phosphate buffer, pH 2.0. [b] C = cyclohexane, D = dioxane, W = water, H = heptane, EA = ethyl acetate, M = methanol, ME = methoxyethanol. [c] HBV = hold-back volume (column solvent retention). [d] Solvent confirmed by nmr. [e] *Anal.* Calcd: F, 10.57. Found: F, 10.90.

Hydrolysis was accomplished in the case of the phthalimidomethyl derivative (III) by simple reflux in methanol using *n*-butylamine as the base.[13] Even more conveniently the trifluoroacetamidomethyl derivative II was easily decomposed by reaction with aqueous base at room temperature similar to the hydrolysis of trifluoroacetyl-protected peptides.[14] In the case of II it was also necessary to heat with dilute acid for a short time to complete the reaction, presumably to hydrolyze the intermediate aminomethyl derivative V which could be detected by paper chromatography but was not isolated. The compounds



exhibited no significant biological activity. For tests used see paper IX.[15]

Experimental Section

Descending paper chromatography was carried out on Whatman No. 1 paper buffered with 0.2 *M* pH 2 phosphate buffer, and rm in a system 1-butanol–phosphate buffer pH 2.0 (2:1). Analyses were prepared by Mr. L. Brancone and staff. Liquid–liquid partition chromatography[16] was carried out on neutral (acid-washed) diatomaceous earth (Celite).

2-Carboxamido-N-phthalimidomethyl-6-demethyl-6-deoxytetracycline.—2-Decarboxamido-2-cyano-6-demethyl-6-deoxytetracycline[2c] (400 mg, 1.0 mmole) was dissolved in 6 ml of concentrated H_2SO_4 at room temperature after which 196 mg (1.1 mmoles) of N-hydroxymethylphthalimide[6] was added with stirring. The solution was stirred at room temperature for 25 min then poured slowly into 200 ml of dry ether with stirring. The precipitated solid was filtered off, washed with ether, and dried. The neutral form was prepared by slurrying the salt in 16 ml of water and adjusting the pH to a constant reading of 5.0 by the addition of 2 *N* aqueous NaOH. The solid was filtered off, washed with water, and dried, 510 mg. The crude product was purified by liquid–liquid partition column chromatography on neutral (acid-washed) diatomaceous earth as indicated in Table I, 240 mg.

In a similar manner, N-hydroxymethylmaleimide[10] and N-hydroxymethyltrifluoroacetamide[9] were treated with the nitrile.

Conversion of 2-Carboxamido-N-phthalimidomethyl-6-demethyl-6-deoxytetracycline to 6-Demethyl-6-deoxytetracycline.—A solution of 58 mg (0.1 mmole) of 2-carboxamido-N-phthalimidomethyl-6-demethyl-6-deoxytetracycline in 25 ml of dry methanol and 0.15 ml of *n*-butylamine was refluxed for 5 hr. The solution was evaporated to dryness and triturated well with dry ether and the solid material was filtered off and dried, 36 mg. Paper chromatography showed no starting material and a new spot corresponding to 6-demethyl-6-deoxytetracycline appeared at R_f 0.72. Turbidometric assay of the crude di-*n*-butylamine salt was 332 μg/ml (tetracycline = 1000).

Conversion of 2-Carboxamido-N-trifluoroacetamidomethyl-6-demethyl-6-deoxytetracycline to 6-Demethyl-6-deoxytetracycline.—A solution of 14 mg (0.025 mmole) of 2-carboxamido-N-trifluoroacetamidomethyl-6-demethyl-6-deoxytetracycline in 0.75 ml (0.075 mmole) of 0.1 *N* aqueous NaOH was allowed to stand at room temperature for 30 min, after which, 4.25 ml of 0.1 *N* methanolic HCl was added, and the resultant solution refluxed for 1 hr. The solution was evaporated to dryness, after which, the residue was dissolved in 0.5 ml of the lower phase of a solvent mixture heptane–ethyl acetate–methoxyethanol–water 160:40:15:4) and brought to pH 5 with solid sodium acetate. This solution was mixed with 1 g of diatomaceous earth and packed on a 10-g diatomaceous earth column moistened with 5 ml of lower phase, and the product was obtained by developing with upper phase; the product, 1.5 mg, being obtained in the third hold-back volume. The product was identified by ultraviolet spectrum and paper chromatography. No attempt was made to establish optimum conditions in the reaction or product isolation.

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(15) M. J. Murrell, Jr., A. S. Ross, and J. H. Boothe, *J. Med. Chem.*, **10**, 359 (1967).

(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).

1,3,2-Diazaphosphorine 2-Oxides. IV.[1] A New Method for the Preparation of 2-(N-Arylamino)- and 2-(N-Alkylamino)-1,3,2-diazaphosphorine 2-Oxides[2]

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Previous articles have been concerned with the synthesis and antitumor activity of compounds of type III.[3] These were prepared by allowing a diamine, N,N′-bis(*para*-substituted benzyl)-1,3-diaminopropane I, to react with an N-arylphosphoramidic dichloride II (Chart I).

Since the synthesis of the N-arylphosphoramidic dichlorides II involves the direct addition of $POCl_3$

(1) Part III: J. H. Billman, J. L. Meisenheimer, and R. F. May, *J. Med. Chem.*, **9**, 772 (1966).

(2) This investigation was supported by Public Health Service Grant CA-06448-03 from the National Institutes of Health, Public Health Service.

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