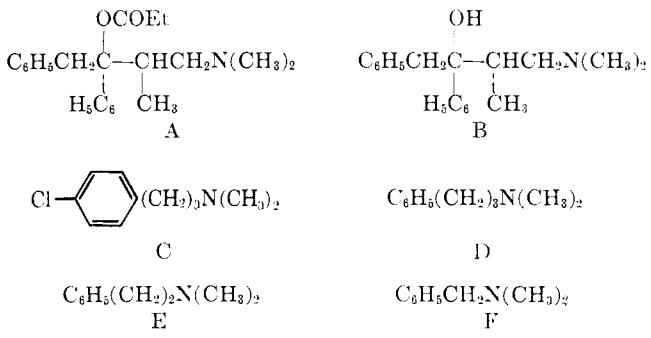


A B C D E F

reason for this may be the relatively little variation in pK_a for these compounds compared to the variation in log P.

	n	r	s	
$\log BR = 0.094 \log P + 0.325$	6	0.782	0.116	(5)
$\log BR = 0.137(pK_a - 9.5) + 0.668$	6	0.248	0.180	(6)
$\log BR = 0.099 \log P + 0.190(pK_a - 9.5) + 0.354$	6	0.853	0.112	(7)

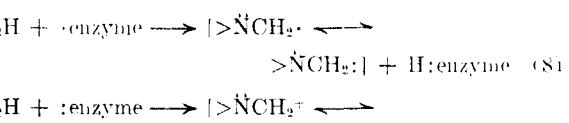
For the work in this report, P is the octanol–water partition coefficient so that the larger the value for P, the more lipophilic the compound. Thus the positive coefficients associated with log P in eq. 2–5 bear out Brodie's idea and McMahon's conclusion that the more lipophilic the compounds are, the more rapidly they are demethylated.

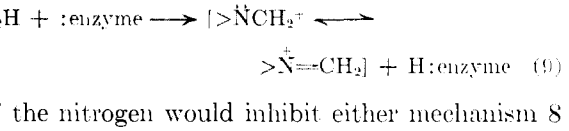
Where electronic terms are significant in the above equations, we note that a negative coefficient is associated with $(pK_a - 9.5)$. This means that the lower the electron density on nitrogen (as measured by $R_3N^+H \rightleftarrows R_3N + H^+$), the greater the demethylation rate. An obvious interpretation of this could be that the lower electron density reduces the chance of protonation of the nitrogen and hence the aqueous solubility of the amine. It must be kept in mind that π or log P is determined[8] so that it is independent of the degree of dissociation; hence π and pK_a are independent variables. Actually, both can play a role in the distribution of the amines between the aqueous and lipophilic phases. The role of pK_a in this process is difficult to assess accurately since it will vary with the pH of the environment and the endobio environment at the sites of action may be different from that of the external solution.

One of the most interesting aspects of McMahon's study is the minor importance of steric effects on the rate of demethylation. One might expect that the demethylation rate of a molecule with such highly branched substituents as *t*-amyl-*t*-butylmethylamine would be so different from *n*-dipropylmethylamine that an equation such as 4 without a steric parameter would give good correlations. It seems to us this would of necessity be true if any group other than a proton were involved in reaction with the nitrogen lone-pair electrons.

The work of Brown[9] has clearly demonstrated that the steric requirements for a proton reacting with highly substituted amines are uniquely low. It is also clear from Brown's work that the steric requirements of other electron-seeking substances such as BH_3, $B(CH_3)_3$, etc. do not parallel those of the proton. The fact that such good correlation is obtained using pK_a, a constant dependent on the steric and electronic requirements of protonation of the amines in question, argues strongly against the electronic term in eq. 4, reflecting action with a function larger than a proton. We interpret this to mean that an enzyme cannot be involved with the lone-pair electrons on nitrogen or those in the N–CH_3 bond in the rate-determining step of demethylation.

An attractive mechanism in which steric influence of the highly branched alkyl group would be at a minimum would be a displacement on hydrogen. Protonation of the nitrogen would inhibit either mechanism 8 or 9 by tying down the lone-pair electrons and thus preventing their stabilization of the intermediates through resonance.





Thus the role of the electron density on nitrogen in the above suggested mechanism for demethylation is complex. If it is very high, the amine is primarily in the ammonium ion form and its lipophilic character is lowered. If it is too low, then the availability of the electrons for stabilizing an intermediate is decreased. In order to test this hypothesis we have derived eq. 10 for compounds 1–18 in Table I. This gives a slightly better correlation than eq. 4. An F test indicates the squared term to be significant at >0.95. Equation 10 does indicate a slightly nonlinear dependence of log BR on the electron density, indicating a possible dual role for the lone-pair electrons. A further analysis of this point using amines having higher and lower electron densities than those studied would be worthwhile.

	r	s	
$\log BR = 0.484 \log P - 0.068(pK_a - 9.5)^2 - 0.267(pK_a - 9.5) - 1.225$	0.924	0.193	(10)

Acknowledgment.—This work was supported by Research Grant GM-07492 from the National Institutes of Health. We wish to thank Edna W. Deutsch for computational assistance.

(9) H. C. Brown, *J. Chem. Educ.*, **36**, 424 (1959).

Alkoxyalkyltetracyclines

C. RICHARD TAMORRIA AND ROBERT C. ESSE

Pharmaceutical Product Development Section Lederle Laboratories Division of American Cyanamid Company, Pearl River, New York

Received April 16, 1965

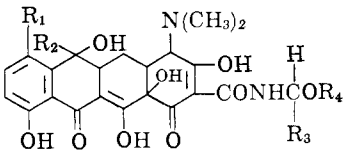
Modifications of tetracyclines involving the carboxamide function have previously been achieved mainly *via* the Mannich or Ritter reactions. Condensations of tetracyclines with formaldehyde and various primary or secondary amines have led to Mannich base deriva-

tives, some of which possess enhanced solubility in water.[1] The Ritter reaction, also used in preparing substituted carboxamide analogs,[2] proceeds *via* the tetracycline nitrile, formed by dehydration of the carboxamide, the nitrile adding to an appropriate olefin to give an alkyl-substituted carboxamide derivative.

We have synthesized a new class of carboxamide-substituted compounds named alkoxyalkyltetracyclines. These compounds were prepared by condensation of a tetracycline antibiotic with an aldehyde and alcohol and have the general structure shown in Table I, where R_1 and R_2 can vary depending on the parent

TABLE I

ALKOXYALKYLTETRACYCLINES



Compd.	R_1	R_2	R_3	R_4
I	H	CH_3	H	CH_3
II	H	CH_3	CH_3	CH_3
III	H	CH_3	C_2H_5	CH_3
IV	Cl	CH_3	H	CH_3
V	Cl	CH_3	CH_3	CH_3
VI	Cl	CH_3	C_2H_5	CH_3

tetracycline employed, while R_3 and R_4 vary with respect to the aldehyde and alcohol used in each synthesis.

The original derivative synthesized, N-(1-methoxy)-methyltetracycline (I), was obtained by refluxing a mixture of tetracycline, formaldehyde, and methanol. The infrared spectrum of I indicated an ether (9.54 μ) and a monosubstituted amide (6.55 μ). As expected, the ultraviolet spectrum [$\lambda_{max}^{0.1\ N\ HCl}$ 218 mμ (ϵ 14,800), 270 (20,000), and 360 (14,400)] was essentially the same as that of tetracycline. Compounds II–VI, prepared in a similar manner, were amorphous but were analytically and paper chromatographically pure. Their infrared and ultraviolet spectra were consistent with the proposed structures. Each of the compounds I–VI had a single, distinct R_f value on paper chromatograms.[3] Crystalline ethylenediamine salts of compounds IV and V were also prepared and were identical with pure IV and V on paper chromatograms.

Biological Data.—In turbidimetric *in vitro* assays against *Staphylococcus aureus*,[4] compounds I–VI varied in activity from 12 to 46% of the parent antibiotic. *In vivo* tests in mice showed the activities to be 6–100% of the parent antibiotic, depending on the particular structure of the compound and mode of administration. The *in vivo* results are given in Table II. Mice infected with *S. aureus* were treated with the test compound, and results are expressed as a decimal fraction of the protection afforded infected mice by the parent antibiotic under the same test conditions. The compounds were tested by intravenous, oral, and subcutaneous routes.

TABLE II

COMPARISON OF *in Vivo* ACTIVITIES OF ALKOXYALKYLTETRACYCLINES *vs.* TETRACYCLINES

Compd.	R_3	Fraction of bioactivity of compd. by various routes[a] i.p.	*p.o.*	s.c.	Parent antibiotic (as HCl)
I	H	0.25	0.062	0.125	Tetracycline
II	CH_3	0.25	0.25–0.50	0.25	Tetracycline
III	C_2H_5	0.25	0.25–0.50	0.50	Tetracycline
IV	H	0.062	0.062	0.062	Chlorotetracycline
V	CH_3	0.062	0.25	0.25	Chlorotetracycline
VI	C_2H_5	0.25	1.00	0.25	Chlorotetracycline

[a] Activity of parent = 1.00.

From Table II it is evident that increasing the size of R_3 from H to C_2H_5 results generally in increased bioactivity. This might be explained by the fact that when R_3 is larger, more facile hydrolysis of the alkoxyalkyltetracycline can occur, yielding the active parent compound. After oral administration, the stomach acidity would be expected to cause even more rapid hydrolysis to the parent tetracycline. This could account for the relatively high potency of VI by the oral route. Chemical evidence also exists indicating that bioactivity is due to breakdown to the parent antibiotic.

In a typical example, I (original turbidimetric assay = 121 γ/mg. *vs.* tetracycline·HCl) was dissolved (5 mg./ml.) in 0.01 *N* HCl. In 2 days the bioassay value doubled. After 2 weeks, a maximum value of 422 γ/mg. was obtained. Concurrently, paper chromatograms showed an increase of tetracycline component and decrease of I.[3]

Another feature of these derivatives is their utility as blocking agents in preventing nitrile formation by dehydration of the carboxamide moiety. In compounds where R_3 is H, it can be shown that agents which normally dehydrate the carboxamide to the nitrile do not affect these analogs. For example, under conditions identical with those used to prepare tetracyclinonitrile from tetracycline (see Experimental Section), I gave no nitrile, as shown by paper chromatography and infrared analysis. Thus, alkoxyalkylation could provide a means of protecting the carboxamide group when it is necessary to treat other portions of the tetracycline molecule with dehydrating agents.

Experimental Section[5]

N-(1-Methoxy)methyltetracycline (I).—Tetracycline (26.64 g., 0.06 mole) was added to a stirred mixture of methanol (360 ml.) and a 46.5% solution of formaldehyde in methanol[6] (120 ml.) and refluxed for 2 hr. The dark amber solution was cooled at 5° for several days. The crystals which formed were filtered and washed successively with cold methanol and cold anhydrous ether. The yield of light yellow crystalline I (methanolate) was 12.7 g. (41%); $\lambda_{max}^{0.1\ N\ HCl}$ 218 mμ (ϵ 14,800), 270 (20,000), and 360 (14,400).

Anal. Calcd. for $C_{25}H_{32}N_2O_{10}$: C, 57.68; H, 6.20; N, 5.38. Found: C, 57.48; H, 5.90; N, 5.19.

(1) W. J. Gottstein, W. F. Minor, and L. C. Cheney, *J. Am. Chem. Soc.*, **81**, 1198 (1959).

(2) C. R. Stephens, Jr., U. S. Patent 3,028,409 (April 3, 1962); *Chem. Abstr.*, **57**, 9769*g* (1962).

(3) The compounds were spotted on papergrams which had previously been treated with a buffer system [0.2 *M* Na_2HPO_4–2.24% citric acid (30:70)] adjusted to pH 3.36, and dried. The papergrams were developed using the following system: nitromethane–benzene–pyridine–Na_2HPO_4–citric acid buffer at pH 3.36 (20:10:3:3), run at 5°.

(4) A. C. Dornbush and E. J. Pelcak, *Ann. N. Y. Acad. Sci.*, **51**, 218 (1948).

(5) The ultraviolet spectra were determined on a Cary Model 11 recording spectrophotometer.

(6) Methyl Formcel is a 46.5% solution of formaldehyde in methanol, obtained from Celanese Corp.

Compounds II–VI were prepared by the following general procedure. The tetracycline was added to a stirred mixture of the alcohol and aldehyde, and the mixture refluxed to give a clear solution. The time in each case was determined by a preliminary small-scale reaction, followed closely by paper chromatography. The solution was allowed to cool, then taken to dryness *in vacuo*, and worked up with anhydrous ether to a solid. Analytical samples were obtained by dissolving the crude solid in chloroform, then washing several times with water. The chloroform, after drying (Na_2SO_4), was evaporated *in vacuo*, and the residue was worked up with anhydrous ether to yield the appropriate alkoxyalkyltetracycline.

N-(1-Methoxy)ethyltetracycline (II).—Tetracycline (4.44 g., 0.01 mole), methanol (75 ml.), and acetaldehyde (25 ml.) were refluxed 2.5 hr. A portion (85%) of the cooled solution, when worked up, gave 1.5 g. of crude II. From the crude material there was obtained 400 mg. of analytically pure II: $\lambda_{max}^{0.1\ N\ HCl}$ 218 mμ (ε 14,800), 270 (19,100), and 360 (12,100).

Anal. Calcd. for $C_{25}H_{30}N_2O_9$: C, 59.75; H, 6.02; N, 5.57. Found: C, 59.64; H, 5.99; N, 5.04, 5.32.

N-(1-Methoxy)propyltetracycline (III).—A mixture of tetracycline (8.88 g., 0.02 mole), methanol (150 ml.), and propionaldehyde (50 ml.) was refluxed 2.5 hr. to give 3.65 g. of crude III. From 2.4 g. of crude material 250 mg. of analytically pure III was obtained: $\lambda_{max}^{0.1\ N\ HCl}$ 218 mμ (ε 15,600), 270 (19,100), and 360 (13,900).

Anal. Calcd. for $C_{26}H_{32}N_2O_9$: C, 60.45; H, 6.25; N, 5.42. Found: C, 59.70; H, 6.39; N, 5.65.

N-(1-Methoxy)methylchlorotetracycline (IV).—A mixture of chlorotetracycline (24.0 g., 0.05 mole), methanol (300 ml.), and a 46.5% solution of formaldehyde in methanol[6] (100 ml.) was refluxed 45 min. Work-up gave 19.3 g. of crude material. From 3.0 g. of crude material, 900 mg. of analytically pure IV was obtained: $\lambda_{max}^{0.1\ N\ HCl}$ 230 mμ (ε 17,200), 268 (18,000), and 370 (9140).

Anal. Calcd. for $C_{24}H_{27}ClN_2O_9$: C, 55.12; H, 5.20; Cl, 6.78; N, 5.36. Found: C, 54.85; H, 5.27; Cl, 7.09; N, 5.27.

N-(1-Methoxy)ethylchlorotetracycline (V).—A mixture of chlorotetracycline (7.2 g., 0.015 mole), methanol (30 ml.), and acetaldehyde (15 ml.) was refluxed for 2.75 hr. Work-up gave 5.47 g. of crude material. From 2.0 g. of crude product, 820 mg. of analytically pure V was obtained: $\lambda_{max}^{0.1\ N\ HCl}$ 230 mμ (ε 17,700), 268 (18,200), and 370 (10,200).

Anal. Calcd. for $C_{25}H_{29}ClN_2O_9$: C, 55.92; H, 5.44; Cl, 6.60; N, 5.22. Found: C, 55.44; H, 5.22; Cl, 6.52; N, 5.22.

N-(1-Methoxy)propylchlorotetracycline (VI).—A mixture of chlorotetracycline (9.6 g., 0.02 mole), methanol (120 ml.), and propionaldehyde (40 ml.) was refluxed 1.5 hr. Work-up yielded 9.7 g. of crude material. From 2.0 g. of crude material there was obtained 850 mg. of analytically pure VI: $\lambda_{max}^{0.1\ N\ HCl}$ 230 mμ (ε 17,600), 266 (18,150), and 370 (10,200).

Anal. Calcd. for $C_{26}H_{31}ClN_2O_9$: C, 56.67; H, 5.67; Cl, 6.44; N, 5.08. Found: C, 56.50; H, 5.81; Cl, 6.50; N, 5.20.

N-(1-Methoxy)methylchlorotetracycline (IV) Ethylenediamine Salt.—To IV (500 mg., 0.96 mmole) was added 14 ml. of a 10% water-in-methanol solution. Triethylamine (0.28 ml.) was added and the solution warmed to 50°. Next 2.0 ml. of an ethylenediamine-in-methanol solution (prepared by adding 1.6 ml. of ethylenediamine to 14.4 ml. of MeOH) was added, and after several minutes of stirring the crystalline salt appeared. After cooling and filtering, the crystals were washed with a 10% water-in-methanol solution, then anhydrous ether, and dried.

Anal. Calcd. for $C_{28}H_{35}ClN_4O_9$: C, 53.56; H, 6.05; Cl, 6.08; N, 9.61. Found: C, 53.20; H, 6.33; Cl, 6.19, 6.23; N, 9.59.

N-(1-Methoxy)ethylchlorotetracycline (V) Ethylenediamine Salt.—Treatment of V, as above, gave the crystalline ethylenediamine salt of V.

Anal. Calcd. for $C_{27}H_{37}ClN_4O_9$: C, 54.31; H, 6.25; Cl, 5.94; N, 9.38. Found: C, 53.95; H, 5.75; Cl, 6.02; N, 9.24.

I and Methanesulfonyl Chloride.—A solution of I (1 g., 1.9 mmoles) in pyridine (10 ml.) was cooled to 0°. To this was slowly added methanesulfonyl chloride (0.3 ml.) while the temperature was kept below 5°. The mixture was stirred 1 hr. at 0–5° and filtered. The filtrate was precipitated into anhydrous ether (40 ml.). The gummy solid obtained was washed several times with anhydrous ether, then worked up by stirring with acetone. A solid was obtained which was shown to be mainly I and some tetracycline. The infrared spectrum showed no nitrile absorption at 4.53 μ.

Under identical conditions, tetracycline was dehydrated at the carboxamide to give tetracycline nitrile as the major product,[7] as demonstrated by paper chromatography and infrared spectrum (absorption at 4.53 μ).

Various Alkoxyalkylation Attempts Followed by Paper Chromatography.—Reactions of tetracyclines with other alcohols and aldehydes were carried out as previously described for I–VI. These were followed by paper chromatography,[3] and in most cases new components were detected on chromatograms. These reaction products were not characterized, but are believed to be alkoxyalkyltetracyclines analogous to I–VI. Those reactions which produced new compounds as demonstrated by paper chromatography are summarized.

Tetracycline and formaldehyde reacted with the following alcohols: ethanol, *n*-butyl alcohol, *t*-butyl alcohol, benzyl alcohol, α-hydroxyacetic acid, 2-phenylethanol, lactic acid, sorbitol, and mannitol. Tetracycline and methanol reacted with the following aldehydes: glyoxylic acid, 2-pyridinecaldehyde, *p*-nitrobenzaldehyde, *p*-chlorobenzaldehyde, 2-furfural, and chloroacetaldehyde. Similarly 6-demethyltetracycline and methanol reacted with formaldehyde and propionaldehyde.

Acknowledgment.—The authors are indebted to Mr. L. Brancone, Mr. W. Fulmor, Mr. A. Dornbush, and Mr. G. Redin and their associates for the microanalyses, ultraviolet analyses, microbiological assays, and biological testing, respectively.

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

Synthesis of Heterocyclic-Substituted Chromones and Related Compounds as Potential Anticancer Agents[1]

DOROTHY DONNELLY, ROSALIE GEOGHEGAN, CONOR O'BRIEN, EVA PHILBIN, AND T. S. WHEELER[2]

Department of Chemistry, University College, Dublin, Ireland

Received June 10, 1965

In continuing previous studies[3] in this laboratory on the synthesis of potential anticancer agents, a further series of heterocyclic-substituted chromones and related compounds has been prepared and submitted for screening under the auspices of the Cancer Chemotherapy National Service Center.

The chromones were synthesized by a standard three-step procedure involving (1) condensation of the appropriate 2-hydroxyacetophenones with heterocyclic acid chlorides to form the esters listed in Table I, (2) Baker–Venkataraman rearrangement[4] of these esters to the corresponding 1,3-diketones listed in Table II, and (3) dehydrative cyclization of the diketones to the corresponding chromones shown in Table III. The diketone, 1-(2-hydroxy-5-methoxyphenyl)-3-(2-quinolyl)propane-1,3-dione, was not isolated in the pure state; Baker–Venkataraman rearrangement of the corresponding ester (II) gave an inseparable mixture of red and white products (pre-

(1) This work was supported by the National Cancer Institute, National Institutes of Health, Bethesda 14, Md.

(2) Deceased.

(3) P. F. Devitt, A. Timoney, and M. A. Vickars, *J. Org. Chem.*, **26**, 4941 (1961).

(4) W. Baker, *J. Chem. Soc.*, 1381 (1933); H. S. Mahal and K. Venkataraman, *Current Sci. (India)*, **2**, 214 (1933); B. G. Doyle, F. Gogan, J. E. Gowan, J. Keane, and T. S. Wheeler, *Sci. Proc. Roy. Dublin Soc.*, **24**, 291 (1948).