tionship exists between the carcinogenic and the antitumor potentialities of the aryl component. Studies by Cook and Kennaway[9] have shown that 9,10-dimethylanthracene is a moderately active carcinogen in mice, whereas benz[*a*]anthracene and 10-methylphenanthrene exhibit no more than slight carcinogenic activity. Since certain methyl-substituted benz[*a*]anthracenes are potent carcinogens, we propose to synthesize the sulfur and nitrogen mustard derivatives of several of these polycyclic aromatic compounds and determine the existence of any carcinogenic–carcinostatic correlations.

Cross-linking ability is generally considered to be a requirement for a tumor-inhibiting alkylating agent. This condition is fulfilled by the bifunctional nitrogen and sulfur mustards. Several of our monofunctional mustards have also displayed pronounced antitumor activity; these have contained a specific polycyclic component, such as a derivative of acridine or anthracene. It is probable that these half-mustards also exert their action by a bifunctional mechanism, one phase of which involves the reaction of the 2-chloroethyl group with the guanine moiety of deoxyribonucleic acid, as shown by Lawley and Wallick,[10] or with some other essential constituent. It seems reasonable that cross-linking can be completed by intercalation of the polycyclic component between the base pairs in deoxyribonucleic acid, a process proposed by Lerman[11] for the acridine nucleus and by Boyland and Green[12] for the polynuclear hydrocarbons.

(9) J. W. Cook and E. L. Kennaway, *Am. J. Cancer*, **39**, 381 (1940); E. L. Kennaway, N. M. Kennaway, and F. L. Warren, *Cancer Res.*, **2**, 157 (1942).

(10) P. D. Lawley and C. A. Wallick, *Chem. Ind.* (London), 633 (1957); P. D. Lawley *Proc. Chem. Soc.*, 290 (1957); P. Brookes and P. D. Lawley, *Brit. Med. Bull.*, **20**, 91 (1964).

(11) L. S. Lerman, *J. Cellular Comp. Physiol.*, **64**, Suppl. 1, 1 (1964).

(12) E. Boyland and B. Green, *Brit. J. Cancer*, **16**, 507 (1962); *J. Mol. Biol.*, **9**, 589 (1964).

# Acetylenic Carbamates. A New Class of Potential Oncolytic Agents

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A series of 1,1-diaryl-2-propynyl carbamates is described. Unlike the previously reported 1,1-dialkyl-2-propynyl carbamates, this series was found to have potent antitumor effects against various tumor systems in mice. Oral as well as parenteral activity of these compounds is demonstrated. Structure–activity relationships are discussed.

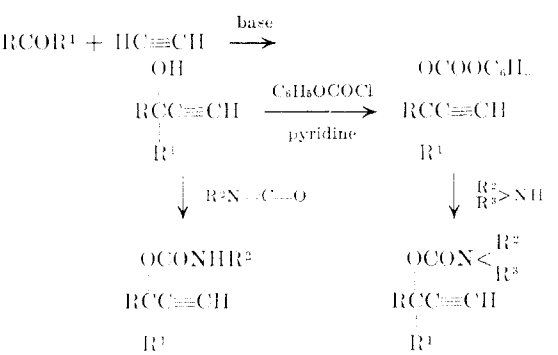
An investigation of the intramolecular reactions of acetylenic compounds[1-3] led to the synthesis of 1,1-diphenyl-2-propynyl carbamate. This compound showed interesting activity in our cancer screen. On this basis, a series of diaryl carbamates was investigated for antitumor activity. In contrast to that reported for 1,1-dialkyl-2-propynyl carbamates,[4,5] these were void of any hypnotic properties. However, unlike the 1,1-dialkyl compounds, this series demonstrated repeatable activity against a series of mouse neoplasms.

Since these compounds were most active against the myelogenous leukemia C1498 and the plasma-cell tumor X5563, these tumors were used to evaluate the relative efficacy of the compounds as antitumor agents. Although most of the carbamates showed activity in these tests, certain variations in structure appeared to promote optimum antitumor effects.

Although the preliminary activity of these compounds was established by the intraperitoneal route of therapy, other routes were explored. The oral activity was of particular interest, inasmuch as it is more desirable to administer oncolytic agents for clinical use by the oral route. Studies were carried out and comparisons made by both the intraperitoneal and oral routes. More extensive studies are in progress including delayed therapy experiments, broad-spectrum tumor studies, tissue localization in mice, hemopoetic effects, and general toxicological effects.

**Chemistry.**—The preparation of the carbamates by a modified procedure described by Mehta and Catlin[4] is outlined in Scheme I. The 1,1-diaryl-2-propyn-1-ols,

Scheme I




prepared by well-known procedures from diaryl ketones, were treated with phenyl chloroformate using pyridine as an acid acceptor in dichloromethane to give the phenyl carbonate intermediate. In the presence of an amine, this intermediate was converted to the carbamate and phenol. The crude products were purified by crystallization to give yields of 5–30%. No effort was made to obtain optimum yields. The N-monosubstituted carbamates could be prepared directly from the 2-propyn-1-ols using an alkyl isocyanate. The allyl carbamates were obtained by catalytic hydrogenation of the appropriately substituted 2-propynyl carbamates.

(1) N. R. Easton and R. D. Dillard, *J. Org. Chem.*, **28**, 2465 (1963).

(2) N. R. Easton, D. R. Cassady, and R. D. Dillard, *ibid.*, **27**, 2927 (1962).

(3) N. R. Easton, D. R. Cassady, and R. D. Dillard, *ibid.*, **29**, 1851 (1964).

(4) M. D. Mehta and E. R. Catlin, U. S. Patent 3,062,870 (Nov 6, 1962).

(5) W. Keil, R. Muschaweck, and E. Rademacher, *Arzneimittel-Forsch.*, **4**, 177 (1954).

All products mentioned above are listed in Table I.

**Pharmacological Methods.**—Because of the obvious physical limitations, only two of the 21 experimental tumor systems maintained in these laboratories were employed for preliminary antitumor testing of this series of compounds. The two systems were selected because of their responsiveness to the carbamates, and the fact that both are used to predict useful candidates for clinical evaluation. Those carbamates showing a high degree of potency against these two systems were then subjected to more extensive studies.

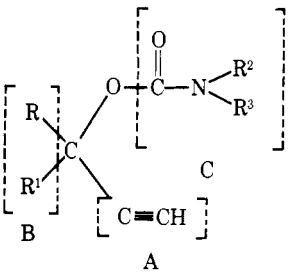
One of the tumors, X5563, is a slow-growing, plasma-cell tumor, the other, an atypical, myelogenous leukemia known as C1498. Procedures for animal-tumor testing in our laboratories have been previously described by Johnson, *et al.*[6] However, in brief, for the solid tumor, a tumor fragment was implanted subcutaneously by trocar in $C_3H$ mice and after 72 hr, treatment was initiated and continued until a total of 10 injections had been given. Two-dimensional measurements were taken after the tenth injection; the activity is reported as a comparison of the tumor sizes of test animals to that of saline controls. A 100% activity would mean that no measurable tumor was present in the test animals at the end of the treatment period. This plasma-cell tumor exhibits some of the same characteristics associated with multiple myeloma in man, including the presence of an abnormal protein found in the serum from animals bearing the disease.

The other test system, the myelogenous leukemia, is maintained in C-57B1/6 mice in the solid form. The test animals are injected with a tumor cell homogenate. Inoculation was by the intraperitoneal route at known, standard-cell concentrations. Inoculated animals usually survive for 14–18 days. Treatment was initiated 24 hr after inoculation, and a total of ten treatments with the compound was given. Activity was determined by prolongation of life of treated animals *vs.* that of saline controls. Those living for 45 days were considered "cured" and were designated as indefinite survivors. These were not calculated in the per cent activity. This tumor system is naturally resistant to many known clinically useful anticancer agents.

The insolubility of the carbamates in most vehicles used for antitumor testing, acacia, saline, sesame oil, and carboxymethylcellulose, did not prevent their effectiveness as antitumor agents. Suspensions of the carbamates using 1:10 dilutions of Emulphor®[7] were used successfully throughout the series.

Prior to testing the compounds against the tumor systems, subacute toxicity studies were conducted using normal Swiss ICR mice. Compounds were administered daily at four different levels for 5 days. Antitumor testing was then initiated at the maximum tolerated level and at two lower levels, usually one-half and one-fourth of the high dose. Dose–response studies on the compounds demonstrating the most potent antitumor properties in the primary screen were then undertaken. In this study, dose levels were given that usually determined the minimum effective dose, and then dosage was increased until evidence of toxicity was seen. The toxic effects were noted by early deaths of the test animals and weight loss as compared to control animals. Visual observations of the general appearance of the animals such as lack of mobility and rough coat were helpful in determining toxicity of the compound tested. The dose levels for maximum antitumor effects were obtained from the dose–response results. These are reported in Table I. For those compounds not included in the extended tests, the best activities indicated by the initial screen are reported.

**Structure–Activity Relationships.**—Antitumor activity against the X5563 and C1498 systems was found for almost all of the 1,1-diaryl-2-propynyl carbamates tested. However, for maximum potency, certain structural requirements were found to be necessary. To help in assessing the importance of each functional portion of the molecule, the carbamate structure was divided into three main areas—the ethynyl (A), the 1,1 substituents (B), and the carbamoyl portion (C)—and the effect of structural changes on antitumor activity is discussed separately for the three areas. The activity discussed here will refer to the results reported in Table I.




The acetylenic group (A) of the molecule was found to be essential to obtain potent antitumor effects. Reduction of this group to the ethylenic derivative gave compounds with much less activity (compare **82** and **83** with **20** and **24**, respectively). Substitution on the terminal position of the triple bond did not appear to alter the antitumor effect significantly (compare **79** and **80** with **4**).

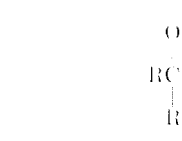
One of the important features of portion B was that R and $R^1$ must both be aromatic groups for the carbamates to show significant antitumor effects. Compounds that deviated from these requirements such as **1**, **2**, and **3** showed little if any effects. When R and $R^1$ were both phenyl, compounds with varying degrees of potencies were obtained depending upon substituents on the remainder of the molecule. Although the effects of substituents on these phenyl groups upon potency were of a lesser degree, certain variations in this area were more beneficial than others. In general, halogen substitution on the phenyl rings gave compounds that demonstrated good antitumor effects. Of the group, chloro, bromo, and iodo, the *p*-chlorophenyl derivatives were the most beneficial in promoting antitumor activity. Of particular interest were the fluorophenyl derivatives (**52**, **54**, **58**, and **59**), since they were the most potent of the halophenyl compounds. Other substitution on the phenyl ring such as methyl, nitro, trifluoromethyl, and phenyl did not seem to enhance activity. When R was phenyl and $R^1$ was heteroaromatic (**72** and **76**), less potent compounds were obtained. However, when $R^1$ was $\alpha$- or $\beta$-naphthyl, potency was retained (**68–70**). The same was true when the R and $R^1$ were joined together forming the fluorenyl moiety (**77** and **78**).

(6) I. S. Johnson, *et al.*, *Cancer Res.*, **20**, 1016 (1960).

(7) Emulphor® is a polyoxyethylated fatty acid available from General Aniline Film Corp., Melrose Park, Ill.

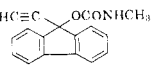
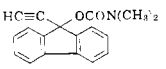
Table I

Acetylenic Carbamates and Their Antitumor Activities



| Compd no. | R | $R^1$ | $R^2$ | $R^3$ | Mp, °C | Formula | %, calcd C | %, calcd H | %, found C | %, found H | Dose,[a] mg/kg | Tumor system X5563 | Tumor system C1498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1[b] | $CH_3$ | $ClCH_2$ | H | H | 66–68[c] | $C_6H_8ClNO_2$ | 44.59 | 4.99 | 44.32 | 5.26 | 60 | 0[d] | . |
| 2 | $C_6H_5$ | $CH_3$ | H | H | 40–42 | $C_{11}H_{11}NO_2$ | 69.82 | 5.85 | 69.98 | 5.89 | 45 | 0 | 0[e] |
| 3 | $C_6H_5$ | H | H | H | 86–88[f] | $C_{10}H_9NO_2$ | 68.56 | 5.18 | 68.43 | 5.19 | 30 | 0 | 0 |
| 4 | $C_6H_5$ | $C_6H_5$ | H | H | 138–140 | $C_{16}H_{13}NO_2$ | 76.47 | 5.22 | 76.48 | 5.49 | 37.5 | 83 (6) | . . . |
| 5[g] | $C_6H_5$ | $C_6H_5$ | $CH_3$ | H | 151–152 | $C_{17}H_{15}NO_2$ | 76.96 | 5.96 | 76.98 | 5.81 | 15 | 64 (10) | 113 (1) |
| 6[g] | $C_6H_5$ | $C_6H_5$ | $C_2H_5$ | H | 108–109 | $C_{18}H_{17}NO_2$ | 77.39 | 6.13 | 77.22 | 6.21 | 30 | 97 (8) | 115 (2) |
| 7 | $C_6H_5$ | $C_6H_5$ | $CH_2CH{=}CH_2$ | H | 96–98 | $C_{19}H_{17}NO_2$ | 78.33 | 5.88 | 78.45 | 6.08 | 18 | 100 (9) | 109 |
| 8 | $C_6H_5$ | $C_6H_5$ | $CH_2C{\equiv}CH$ | H | 125–127 | $C_{19}H_{15}NO_2$ | 78.87 | 5.22 | 78.91 | 5.31 | 15 | 98 (8) | 84 |
| 9 | $C_6H_5$ | $C_6H_5$ | Cyclopropyl | H | 118–120 | $C_{19}H_{17}NO_2$ | 78.33 | 5.88 | 78.60 | 6.07 | 15 | 0 | 39 |
| 10 | $C_6H_5$ | $C_6H_5$ | $CH_2CH_2OH$ | H | 110–112 | $C_{18}H_{17}NO_2$ | 73.20 | 5.80 | 73.24 | 6.04 | 15 | 0 | 0 |
| 11 | $C_6H_5$ | $C_6H_5$ | $CH_2C_6H_5$ | H | 103–105 | $C_{23}H_{19}NO_2$ | 80.91 | 5.60 | 81.16 | 5.84 | 30 | 0 | 0 |
| 12 | $C_6H_5$ | $C_6H_5$ | $CH_2CH_2C_6H_5$ | H | 121–123 | $C_{24}H_{21}NO_2$ | 81.10 | 5.95 | 81.10 | 6.08 | 60 | 47 (9) | 20 |
| 13 | $C_6H_5$ | $C_6H_5$ | Cyclopentyl | H | 142–145 | $C_{21}H_{21}NO_2$ | 78.97 | 6.63 | 78.69 | 6.75 | 15 | 67 (6) | 157 (3) |
| 14 | $C_6H_5$ | $C_6H_5$ | Cyclohexyl | H | 160–161 | $C_{22}H_{23}NO_2$ | 79.25 | 6.95 | 79.40 | 7.12 | 7.5 | 96 (7) | 183 |
| 15 | $C_6H_5$ | $C_6H_5$ | Cycloheptyl | H | 162–165 | $C_{23}H_{25}NO_2$ | 79.50 | 7.25 | 79.63 | 7.48 | 15 | 100 (7) | 138 |
| 16 | $C_6H_5$ | $C_6H_5$ | Cyclooctyl | H | 148–150 | $C_{24}H_{27}NO_2$ | 79.74 | 7.53 | 79.47 | 7.48 | 30 | 100 (7) | 77 (1) |
| 17 | $C_6H_5$ | $C_6H_5$ | $CH_2CH_2CH_2N(CH_3)_2$ | H | 98–100 | $C_{21}H_{24}N_2O_2$ | 74.97 | 7.19 | 74.91 | 7.47 | 15 | 62 (7) | 45 |
| 18[g] | $C_6H_5$ | $C_6H_5$ | 4-$ClC_6H_4$ | H | 89–90 | $C_{22}H_{16}ClNO_2$ | 73.02 | 4.45 | 72.87 | 4.51 | 150 | 30 (10) | 36 |
| 19 | $C_6H_5$ | $C_6H_5$ | $NH_2$ | H | 107–109 | $C_{16}H_{14}N_2O_2$ | 72.16 | 5.29 | 72.10 | 5.46 | 30 | 0 | 20 |
| 20 | $C_6H_5$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 102–104 | $C_{18}H_{17}NO_2$ | 77.39 | 6.13 | 77.58 | 6.18 | 15 | 65 (10) | 68 |
| 21 | $C_6H_5$ | $C_6H_5$ | $-(CH_2)_4-$ | | 164–166 | $C_{20}H_{19}NO_2$ | 78.66 | 6.27 | 78.90 | 6.53 | 15 | 100 (8) | 133 |
| 22 | $C_6H_5$ | $C_6H_5$ | $-CH_2CH_2OCH_2CH_2-$ | | 143–144 | $C_{20}H_{19}NO_3$ | 74.74 | 5.96 | 74.92 | 6.32 | 15 | 99 (9) | 74 |
| 23 | $C_6H_5$ | $C_6H_5$ | $-(CH_2)_5-$ | | 158–160 | $C_{21}H_{21}NO_2$ | 78.97 | 6.63 | 78.81 | 6.79 | 150 | . . . | 0 |
| 24 | 4-$ClC_6H_4$ | $C_6H_5$ | H | H | 140–142 | $C_{16}H_{12}ClNO_2$ | 67.25 | 4.23 | 67.27 | 4.41 | 40 | 100 (9) | 101 |
| 25[g] | 4-$ClC_6H_4$ | $C_6H_5$ | $CH_3$ | H | 153–155 | $C_{17}H_{14}ClNO_2$ | 68.11 | 4.70 | 68.26 | 4.74 | 30 | 96 (8) | 69 (1) |
| 26 | 4-$ClC_6H_4$ | $C_6H_5$ | $CH_2CH{=}CH_2$ | H | 106–109 | $C_{19}H_{16}ClNO_2$ | 70.04 | 4.95 | 69.86 | 5.24 | 30 | 87 (2) | 182 (2) |
| 27 | 4-$ClC_6H_4$ | $C_6H_5$ | Cyclohexyl | H | 156 | $C_{22}H_{22}ClNO_2$ | 71.82 | 6.03 | 71.89 | 6.02 | 75 | 100 (5) | 49 (7) |
| 28 | 4-$ClC_6H_4$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 135–137 | $C_{18}H_{16}ClNO_2$ | 68.90 | 5.13 | 68.91 | 5.41 | 30 | 100 (8) | 62 |
| 29 | 4-$ClC_6H_4$ | $C_6H_5$ | $-(CH_2)_4-$ | | 90–92 | $C_{20}H_{18}ClNO_2$ | 70.69 | 5.33 | 70.31 | 5.88 | 30 | 94 (10) | 126 (7) |
| 30 | 3-$ClC_6H_4$ | $C_6H_5$ | H | H | 113–115 | $C_{16}H_{12}ClNO_2$ | 67.25 | 4.23 | 67.09 | 4.41 | 60 | 0 | 0 |
| 31[g] | 3-$ClC_6H_4$ | $C_6H_5$ | $CH_3$ | H | 141–142 | $C_{17}H_{14}ClNO_2$ | 68.11 | 4.70 | 67.96 | 4.92 | 90 | 100 (7) | 98 |
| 32 | 3-$ClC_6H_4$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 97–99 | $C_{18}H_{16}ClNO_2$ | 68.90 | 5.13 | 68.62 | 5.01 | 90 | 100 (9) | 76 |
| 33 | 2-$ClC_6H_4$ | $C_6H_5$ | H | H | 169–171 | $C_{16}H_{12}ClNO_2$ | 67.25 | 4.23 | 67.48 | 4.49 | 90 | 82 (5) | 0 |
| 34 | 2-$ClC_6H_4$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 154–156 | $C_{18}H_{16}ClNO_2$ | 68.90 | 5.13 | 68.91 | 5.12 | 90 | 65 (7) | 21 |
| 35 | 4-$ClC_6H_4$ | 4-$ClC_6H_4$ | H | H | 131–133 | $C_{16}H_{11}Cl_2NO_2$ | 60.02 | 3.46 | 60.19 | 3.66 | 60 | 93 (6) | 60 |
| 36[g] | 4-$ClC_6H_4$ | 4-$ClC_6H_4$ | $CH_3$ | H | 173–174 | $C_{17}H_{13}Cl_2NO_2$ | 61.09 | 3.92 | 60.98 | 3.91 | 24 | 100 (9) | 111 (1) |
| 37 | 4-$ClC_6H_4$ | 4-$ClC_6H_4$ | $CH_3$ | $CH_3$ | 162–164 | $C_{18}H_{15}Cl_2NO_2$ | 62.08 | 4.34 | 62.16 | 4.59 | 60 | 100 (8) | 84 |
| 38 | 3,4-$Cl_2C_6H_3$ | $C_6H_5$ | H | H | 167–169 | $C_{16}H_{11}Cl_2NO_2$ | 60.02 | 3.46 | 60.34 | 3.64 | 150 | 24 (10) | 0 |
| 39 | 3,4-$Cl_2C_6H_3$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 157–159 | $C_{18}H_{15}Cl_2NO_2$ | 62.08 | 4.34 | 62.19 | 4.50 | 150 | 74 (8) | 0 |
| 40 | 3,4-$Cl_2C_6H_3$ | $C_6H_5$ | $-(CH_2)_4-$ | | 121–123 | $C_{20}H_{17}Cl_2NO_2$ | 64.18 | 4.57 | 64.25 | 4.81 | 150 | . . . | 26 |
| 41 | 2,4-$Cl_2C_6H_3$ | $C_6H_5$ | H | H | 163–165 | $C_{16}H_{11}Cl_2NO_2$ | 60.02 | 3.46 | 60.28 | 3.70 | 300 | 0 | 0 |
| 42 | 2,4-$Cl_2C_6H_3$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 157–159 | $C_{18}H_{15}Cl_2NO_2$ | 62.08 | 4.34 | 62.31 | 4.49 | 150 | . | 0 |
| 43 | 3,4-$Cl_2C_6H_3$ | 4-$ClC_6H_4$ | H | H | 154–156 | $C_{16}H_{10}Cl_3NO_2$ | 54.19 | 2.84 | 54.42 | 3.01 | 150 | 58 (10) | 0 |
| 44 | 2,4-$Cl_2C_6H_3$ | 4-$ClC_6H_4$ | H | H | 145–146 | $C_{16}H_{10}Cl_3NO_2$ | 54.19 | 2.84 | 54.35 | 3.01 | 60 | 0 | 0 |
| 45 | 4-$BrC_6H_4$ | $C_6H_5$ | H | H | 141–143 | $C_{16}H_{12}BrNO_2$ | 58.20 | 3.66 | 58.39 | 3.83 | 60 | 95 (9) | 120 (1) |
| 46[g] | 4-$BrC_6H_4$ | $C_6H_5$ | $CH_3$ | H | 149–151 | $C_{17}H_{14}BrNO_2$ | 59.32 | 4.09 | 59.50 | 4.30 | 60 | 100 (8) | 82 (2) |
| 47 | 4-$BrC_6H_4$ | $C_6H_5$ | Cyclohexyl | H | 160–162 | $C_{22}H_{22}BrNO_2$ | 64.08 | 5.37 | 64.13 | 5.41 | 150 | 39 (3) | 145 |
| 48 | 4-$BrC_6H_4$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 147–149 | $C_{18}H_{16}BrNO_2$ | 60.35 | 4.50 | 60.64 | 4.61 | 30 | 87 (8) | 71 |
| 49 | 4-$BrC_6H_4$ | $C_6H_5$ | $-(CH_2)_4-$ | | 102–104 | $C_{20}H_{18}BrNO_2$ | 62.51 | 4.72 | 62.58 | 4.79 | 30 | 100 (9) | 56 |
| 50 | 3-$BrC_6H_4$ | $C_6H_5$ | H | H | 127–129 | $C_{16}H_{12}BrNO_2$ | 58.20 | 3.66 | 58.39 | 3.80 | 100 | . . | 37 |
| 51 | 3-$BrC_6H_4$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 111–113 | $C_{18}H_{16}BrNO_2$ | 60.35 | 4.50 | 60.42 | 4.61 | 150 | . | 71 |
| 52 | 4-$FC_6H_4$ | $C_6H_5$ | H | H | 99–101 | $C_{16}H_{12}FNO_2$ | 71.36 | 4.49 | 71.46 | 4.27 | 50 | 100 (8) | 107 (1) |
| 53 | 4-$FC_6H_4$ | $C_6H_5$ | $CH_2C{\equiv}CH$ | H | 121–123 | $C_{19}H_{14}FNO_2$ | 74.25 | 4.59 | 74.48 | 4.59 | 15 | 50 (10) | 85 (1) |
| 54 | 4-$FC_6H_4$ | $C_6H_5$ | Cyclohexyl | H | 168–170 | $C_{22}H_{22}FNO_2$ | 75.19 | 6.31 | 75.43 | 6.44 | 10 | 100 (2) | 98 (1) |
| 55 | 4-$FC_6H_4$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 121–123 | $C_{18}H_{16}FNO_2$ | 72.71 | 5.42 | 72.77 | 5.42 | 15 | 100 (10) | 207 (6) |
| 56 | 4-$FC_6H_4$ | $C_6H_5$ | $-(CH_2)_4-$ | | 131–133 | $C_{20}H_{18}FNO_2$ | 74.28 | 5.61 | 74.43 | 5.84 | 20 | 100 (9) | 143 (6) |
| 57 | 4-$FC_6H_4$ | 4-$FC_6H_4$ | H | H | 121–123 | $C_{16}H_{11}F_2NO_2$ | 66.90 | 3.86 | 66.91 | 4.14 | 35 | 100 (7) | 94 (3) |
| 58 | 4-$FC_6H_4$ | 4-$FC_6H_4$ | Cyclohexyl | H | 177–179 | $C_{22}H_{21}F_2NO_2$ | 71.53 | 5.72 | 71.52 | 5.93 | 5 | 100 (8) | 106 |
| 59 | 4-$FC_6H_4$ | 4-$FC_6H_4$ | $CH_3$ | $CH_3$ | 144–146 | $C_{18}C_{16}F_2NO_2$ | 68.56 | 4.80 | 68.52 | 4.92 | 15 | 100 (9) | 100 |
| 60 | 4-$FC_6H_4$ | 4-$FC_6H_4$ | $-(CH_2)_4-$ | | 143–145 | $C_{20}H_{17}F_2NO_2$ | 70.37 | 5.01 | 70.67 | 5.39 | 30 | 100 (6) | 0 |
| 61 | 4-$IC_6H_4$ | $C_6H_5$ | H | H | 144–146 | $C_{16}H_{12}INO_2$ | 50.95 | 3.20 | 51.22 | 3.35 | 45 | 100 (10) | 106 |
| 62 | 4-$CF_3C_6H_4$ | $C_6H_5$ | H | H | 143–145 | $C_{17}H_{12}F_3NO_2$ | 63.95 | 3.79 | 63.86 | 3.85 | 150 | . . . | 0 |
| 63 | 4-$CF_3C_6H_4$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 176–178 | $C_{19}H_{16}F_3NO_2$ | 65.70 | 4.64 | 65.84 | 4.81 | . . . | . . . | . . |
| 64 | 4-$O_2NC_6H_4$ | $C_6H_5$ | H | H | 165–167 | $C_{16}H_{12}N_2O_4$ | 64.86 | 4.08 | 65.02 | 4.14 | 60 | 0 | . . . |
| 65 | 4-$CH_3C_6H_4$ | $C_6H_5$ | H | H | 128–130 | $C_{17}H_{15}NO_2$ | 76.96 | 5.70 | 76.84 | 5.41 | 15 | 0 | 0 |
| 66 | 4-$C_6H_5C_6H_4$ | $C_6H_5$ | H | H | 154–156 | $C_{22}H_{17}NO_2$ | 79.98 | 5.43 | 80.32 | 5.17 | 45 | . . . | 141 (3) |
| 67 | 4-$C_6H_5C_6H_4$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 145–147 | $C_{24}H_{21}NO_2$ | 81.10 | 5.95 | 81.16 | 5.99 | 60 | . . . | 81 |
| 68 | 2-$C_{10}H_7$ | $C_6H_5$ | H | H | 163–165 | $C_{20}H_{15}NO_2$ | 79.71 | 5.02 | 79.88 | 5.03 | 12.5 | 100 (5) | 85 |
| 69 | 2-$C_{10}H_7$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 111–113 | $C_{22}H_{19}NO_2$ | 80.21 | 5.81 | 80.11 | 5.95 | 30 | 100 (8) | 164 (7) |
| 70 | 2-$C_{10}H_7$ | $C_6H_5$ | $-(CH_2)_4-$ | | 83–85 | $C_{24}H_{23}NO_2$ | 81.10 | 5.95 | 81.21 | 6.12 | 45 | 100 (7) | 151 (1) |
| 71 | 1-$C_{10}H_7$ | $C_6H_5$ | H | H | 157–159 | $C_{20}H_{15}NO_2$ | 79.71 | 5.02 | 79.98 | 5.09 | 30 | 100 (5) | 72 |
| 72 | 2-$C_5H_4N$ | $C_6H_5$ | H | H | 134–135 | $C_{18}H_{12}N_2O_2$ | 71.41 | 4.80 | 71.80 | 5.16 | 150 | 100 (2) | 0 |
| 73[g] | 2-$C_5H_4N$ | $C_6H_5$ | $CH_3$ | H | 112–115 | $C_{19}H_{14}N_2O_2$ | 72.46 | 5.30 | 72.20 | 5.60 | 60 | 92 (7) | 0 |
| 74[g] | 2-$C_5H_4N$ | $C_6H_5$ | $C_6H_5$ | H | 119–120 | $C_{24}H_{18}N_2O_2$ | 76.81 | 4.91 | 76.88 | 5.02 | . . . | . . . | . . |
| 75 | 2-$C_5H_4N$ | $C_6H_5$ | $CH_3$ | $CH_3$ | 121–125 | $C_{17}H_{18}N_2O$ | 72.84 | 5.75 | 72.85 | 6.00 | 60 | 50 (8) | 0 |
| 76 | 2-$C_4H_3S$ | $C_6H_5$ | H | H | 114–115 | $C_{15}H_{13}NO_2S$ | 66.39 | 4.82 | 66.59 | 4.92 | 300 | 0 | 0 |

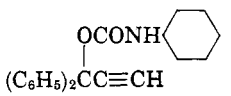
TABLE I (*Continued*)

| Compd no. | Structure | Mp, °C | Formula | —% calcd— C | H | —% found— C | H | Dose,[a] mg/kg | Tumor system X5563 | C1498 |
|---|---|---|---|---|---|---|---|---|---|---|
| 77[g] |  | 153–154 | $C_{11}H_{13}NO_2$ | 77.55 | 4.97 | 77.26 | 5.13 | 10 | 47 (10) | 29 |
| 78 |  | 182–183 | $C_{16}H_{15}NO_2$ | 77.96 | 5.45 | 78.24 | 5.57 | 18 | 100 (8) | 77 |
| 79 | $(C_6H_5)_2CC{\equiv}CCH_3$ <br> OCONH$_2$ | 147–149 | $C_{17}H_{15}NO_2$ | 76.96 | 5.96 | 76.74 | 5.65 | 60 | 27 (10) | 28 |
| 80 | $(C_6H_5)_2CC{\equiv}CBr$ <br> OCSNHCH$_3$ | 150–152 | $C_{16}H_{12}BrNO_2$ | 58.20 | 3.66 | 58.89 | 3.69 | 45 | 80 (6) | 66 |
| 81 | $(C_6H_5)_2CC{\equiv}CH$ <br> OCON(CH$_3$)$_2$ | 135–136 | $C_{17}H_{15}NO_2$ | *h* | | | | 60 | 25 (10) | 0 |
| 82 | $(C_6H_5)_2CCH{=}CH_2$ <br> OCONH$_2$ | 102–104 | $C_{15}H_{13}NO_2$ | 76.84 | 6.81 | 77.21 | 6.83 | 30 | 43 (6) | 0 |
| 83 | *p*-ClC$_6$H$_4$C(C$_6$H$_5$)CH=CH$_2$ <br> OH | 111–113 | $C_{14}H_{14}ClNO_2$ | 66.68 | 4.90 | 66.97 | 5.03 | 7.5 | 0 | 39 |
| 84 | *p*-ClC$_6$H$_4$C(C$_6$H$_5$)C≡CH <br> OCOOC$_6$H$_5$ | 47–48 | $C_{15}H_{11}ClO$ | 74.23 | 4.56 | 74.23 | 4.86 | 60 | 0 | 0 |
| 85[i] | $(C_6H_5)_2CC{\equiv}CH$ | 76–78 | $C_{29}H_{16}O_3$ | 80.47 | 4.91 | 80.75 | 5.20 | 60 | 35 (9) | 0 |

[a] A total of ten treatments were given, by daily injections intraperitoneally, at each dose level indicated, and the reported activities of these compounds against the two systems are the results of a specific dose–response test for each compound and should be considered in a qualitative manner in comparing relative potencies. Activity must be greater than 20% to be considered significant. Myleran at 30 mg/kg inhibited the X5563 system 100% and did not inhibit the C1498 system. $HN_2$ at 0.15 mg/kg did not inhibit the X5563 system but gave a 34% prolongation of life against the C1498 system. Cytoxan (cyclophosphamide) at 50 mg/kg inhibited the X5563 system 100% and the C1498 system 39%. [b] Those compounds with $R^2$ and $R^3$ being hydrogen were made by method A as described in the Experimental Section. The compounds with one or both $R^2$ and $R^3$ being alkyl were prepared by method B. [c] Lit.[4] mp 66–68°. [d] The number given is the per cent inhibition of the tumor, and the number in parentheses indicates total survivors out of ten at the end of the treatment period. [e] The number given is the per cent prolongation of life of treated animals *vs.* saline controls and the number in parentheses is the number of animals surviving for 45 days from date of inoculation and are considered cures. These animals are not calculated in the per cent activity. [f] W. Logemann, *et al.* [*Farmaco* (Pavia), *Ed. Sci.*, **8**, 406 (1953)] report mp 87°. [g] Made by method C as described in Experimental Section. [h] *Anal.* Calcd: N, 4.98. Found: N, 5.18. [i] Intermediate described in method A recrystallized from benzene–petroleum ether (bp 35–60°).

TABLE II

INTRAPERITONEAL *vs.* ORAL TREATMENT USING COMPOUND **14**



| Tumor system | Dose level, mg/kg[a] | Route of admin | Av wt change, T/C | % activity | Indefinite survivors |
|---|---|---|---|---|---|
| C1498 | 7.5 | Ip | −1.5/1.9 | 61 | 0 |
| | 10.0 | Ip | −0.4/1.9 | 83 | 1 |
| | 12.0 | Ip | −2.8/1.9 | 98 | 3 |
| | 15.0 | Ip | −2.7/1.9 | 0 | 7 |
| | 15.0 | Oral | −3.6/0.5 | 107 | 1 |
| | 20.0 | Oral | −2.6/0.5 | 99 | 2 |
| | 25.0 | Oral | −3.7/0.5 | 35 | 4 |
| X5563 | 7.5 | Ip | −0.9/2.0 | 92 | |
| | 10.0 | Ip | −1.2/2.0 | 95 | |
| | 12.0 | Ip | −3.1/2.0 | 100 | |
| | 15.0 | Oral | −2.0/1.2 | 97 | |
| | 20.0 | Oral | −2.2/1.2 | 100 | |
| | 25.0 | Oral | −2.7/1.2 | 100 | |

[a] The dose was administered once daily for 10 days.

Definite requirements also have been demonstrated for the "C" portion of the carbamates. If this portion was hydrogen, *e.g.*, the 1,1-diaryl-2-propyn-1-ols, no significant antitumor effect was found. This was true for the phenylcarbonate intermediate **85** and the thiocarbamate **81**. It could therefore be concluded that the carbamoyl portion O=CN< is necessary to produce compounds with significant activity.

A comparison of the $R^2$ and $R^3$ substituents revealed that they could be varied widely with high potency being maintained. These variations included compounds where $R^2$ and $R^3$ were hydrogen, the N-methyl, N-allyl, N-propynyl, and N,N-dimethyl derivatives, and compounds where the substituents with the nitrogen formed a pyrrolidine ring. However, the N-phenyl derivative (**18**) was much less effective.

When $R^2$ was hydrogen and $R^3$ cyclohexyl (**14**), very high potency was found. This group was particularly effective when R and $R^1$ were *p*-fluorophenyl (**58**). Also, other N-cycloalkyl groups such as cyclopentyl, cycloheptyl, and cyclooctyl (**13**, **15**, and **16**) gave compounds of high potency.

**Intraperitoneal *vs.* Oral Treatment.**—In Table II, a comparison of intraperitoneal and oral administration of compound **14** against the X5563 and C1498 systems is given. In both systems, it appears that an equivalent antitumor effect can be seen using oral doses approximately twice that used by the intraperitoneal route. Other carbamates have also demonstrated this type of oral activity.

## Experimental Section[8]

**1,1-Diaryl-2-propyn-1-ols** were prepared by known procedures[9] from the appropriately substituted benzophenones and were

(8) All melting points were determined using a Mel-Temp melting point apparatus and are uncorrected.

(9) K. Campbell, B. Campbell, and L. Eby, *J. Am. Chem. Soc.*, **60**, 2882 (1938).

purified by distillation or crystallization. In some cases, the crude products were used.

**1,1-Diaryl-2-propynyl N,N-Disubstituted Carbamates.** The following specific examples represent the three methods used for making the carbamates listed in Table I. They are designated as methods A, B, and C.

**Method A. 1-(4-Bromophenyl)-1-phenyl-2-propynyl Carbamate.** A solution of 57.4 g (0.2 mole) of 1-(4-bromophenyl)-1-phenyl-2-propyn-1-ol and 40 ml of pyridine in 200 ml of $CH_2Cl_2$ was cooled to 0° and 31.3 g (0.2 mole) of phenyl chloroformate was added dropwise with stirring over 30 min. Stirring and cooling were continued for 4 hr. Ice water (200 ml) and 500 ml of ether were added, and the organic layer was separated and washed with excess 5% HCl, saturated $NaHCO_3$, and water. After drying ($MgSO_4$), the solution was added dropwise to 400 ml of liquid $NH_3$ over a 15-min period. The resulting mixture was stirred for 16 hr and washed with 5% NaOH solution. After drying over $MgSO_4$, the solvent was removed and the residue was crystallized from benzene–petroleum ether (bp 35–60°). A white, crystalline solid was obtained: yield 6.5 g (25%) (see Table I, **45**).

**Method B. 1-(4-Bromophenyl)-1-phenyl-2-propynyl 1-Pyrrolidinecarboxylate.** The same procedure was used as above to make the phenyl carbonate intermediate on a 0.1 mole scale. The ether $CH_2Cl_2$ solution of the intermediate was added to 50 ml of pyrrolidine in an equal volume of ether, and the mixture was stirred for 16 hr at room temperature and washed with excess 5% HCl then 5% NaOH solution. After drying, the solvent was removed at reduced pressure. The residue was crystallized from benzene–petroleum ether in a yield of 20% (Table I, **49**).

**Method C. 1,1-Diphenyl-2-propynyl N-Methylcarbamate.** A solution of 0.1 mole of 1,1-diphenyl-2-propyn-1-ol, 0.25 mole of methyl isocyanate, and 1 g of triethylenediamine in 200 ml of $CHCl_3$ was left for 4 days at room temperature. All of the low-boiling materials were removed at reduced pressure and the residue was recrystallized twice from benzene–petroleum ether. The product was obtained in a 30% yield (Table I, **5**).

**1,1-Diphenylallyl N,N-dimethylcarbamate.** A solution of 27.9 g of 1,1-diphenyl-2-propynyl N,N-dimethylcarbamate in 200 ml of 4:1 benzene–petroleum ether (bp 85–100°) was hydrogenated at 40 psi using 0.5 g of 5% Pd–$BaSO_4$ and 0.5 g of KOH until 1 equiv of hydrogen was taken up. The catalyst was filtered and the solvent was removed at reduced pressure. The residue was crystallized twice from petroleum ether; yield 27% (Table I, **82**).

In a similar manner, 1-(4-chlorophenyl)-1-phenyl-2-propynyl carbamate was hydrogenated to **1-(4-chlorophenyl)-1-phenylallyl carbamate** (Table I, **83**).

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# The 6-Deoxytetracyclines. VII. Alkylated Aminotetracyclines Possessing Unique Antibacterial Activity

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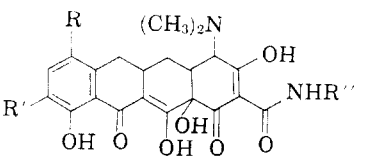
The reductive methylation of 7-nitro-6-demethyl-6-deoxytetracycline gives 7-dimethylamino-6-demethyl-6-deoxytetracycline (VI). Similarly, reductive alkylation of other nitro- or aminotetracyclines forms related derivatives. An *in vitro* spectrum of VI is presented and its unique activity against tetracycline-resistant staphylococci is discussed.

Nitration of 6-demethyl-6-deoxytetracycline (I) in strong acid results in electrophilic substitution at the 7 and 9 positions.[1] Subsequent reactions of these substances to form amino, diazonium, and further transformation products have been the subject of previous papers from these and other laboratories.[2]

In the course of new investigations into the chemistry of these modified antibiotics in the hope of further enhancing the pronounced antibacterial activity possessed by several members of this series, or to find new types of antibacterial activity (*i.e.*, broadened spectrum of activity) we had occasion to examine the reductive alkylation of these substances.

Reductive methylation of 7- or 9-nitro- (II or III) or -amino-6-demethyl-6-deoxytetracycline (IV or V) in methoxyethanol under restricted pH conditions using 10% palladium-on-charcoal catalyst at atmospheric pressure, gave 7- or 9-dimethylamino-6-demethyl-6-deoxytetracycline (VI or VII) and their 4-epimers. These compounds could be purified using liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth. The reaction was




I, R = R′ = R″ = H
II, R = $NO_2$; R′ = R″ = H
III, R = R″ = H; R′ = $NO_2$
IV, R = $NH_2$; R′ = R″ = H
V, R = R″ = H; R′ = $NH_2$
VI, R = $(CH_3)_2N$; R′ = R″ = H
VII, R = R″ = H; R′ = $(CH_3)_2N$
VIII, R = $(CH_3)_2N$; R′ = H, R″ = $CH_2N$
IX, R = $(C_2H_5)_2N$; R′ = R″ = H

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

(2) J. J. Hlavka, A. Sehneller, H. Krazinski, and J. H. Boothe, *ibid.*, **84**, 1426 (1962); J. J. Hlavka, H. Krazinski, and J. H. Boothe, *J. Org. Chem.*, **27**, 3674 (1962); J. L. Spencer, J. J. Hlavka, J. Petisi, H. M. Krazinski, and J. H. Boothe, *J. Med. Chem.*, **6**, 405 (1963); J. J. Hlavka and H. M. Krazinski, *J. Org. Chem.*, **28**, 1422 (1963); C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. Schach, von Wittenau, *J. Am. Chem. Soc.*, **85**, 2643 (1963).