tinuous stirring was employed and the reaction was carried out in an ice–salt bath. The mixture was poured into 100 ml of ice and water with stirring. After standing overnight, the products were isolated as solids or as oils which crystallized on standing. Recrystallization gave the compounds listed in Table I. In addition to these compounds several known compounds[3] of this type were prepared by the same procedure. Alternately the pyridine was added slowly to a cooled mixture of the aniline and *p*-toluenesulfonyl chloride.

N,N-Bis(2-fluoroethyl)anilines (II).—A mixture of 0.01 mole of N,N-bis(2-*p*-toluenesulfonyloxyethyl)aniline (V) and 0.1 mole of anhydrous KF in approximately 50 ml of anhydrous solvent was heated on a steam bath with stirring for 4–24 hr. The reaction mixture was cooled and, after removal of any precipitated potassium tosylate, poured into 200 ml of ice water. After standing the product was obtained by filtration or extraction. The compounds reported in Table II were then obtained by recrystallization, distillation, or alumina chromatography. When the solvent used was methanol, ethanol, or 2,2′-oxydiethanol products of the type included in Table III were sometimes isolated. With DMF as solvent, V (R = 3-F) gave morpholine X, bp 100–105° (0.2 mm), in 66% yield. The hydrochloride of X had mp 166–167° (from acetone–ether).

Anal. Calcd for $C_{10}H_{13}ClFNO$: C, 55.30; H, 5.98; N, 6.44; Cl, 16.32. Found: C, 55.25; H, 5.87; N, 6.55; Cl, 16.51.

Also with DMF as solvent and V (R = 2-Cl), a morpholine, bp 102–105° (0.3 mm), was obtained in 50% yield. This material solidified, mp 62–64°.

Anal. Calcd for $C_{10}H_{13}ClNO$: C, 60.70; H, 6.09; N, 7.11; Cl, 18.00. Found: C, 60.88; H, 5.94; N, 7.06; Cl, 17.75.

The hydrochloride had mp 134–136° (from acetone–ether).

Anal. Calcd for $C_{10}H_{13}Cl_2NO$: C, 51.30; H, 5.59; N, 5.98; Cl, 30.28. Found: C, 51.28; H, 5.62; N, 5.98; Cl, 30.19.

In addition to the procedure noted above II (R = H and R = 3-Cl) was also prepared as follows. A mixture of 21.8 g (0.1 mole) of N,N-bis(2-chloroethyl)aniline (IV) in 250 ml of absolute methanol and 58 g (1.0 mole) of anhydrous KF was refluxed with stirring for 8 hr. The mixture was cooled to room temperature and poured with stirring into cold water. The mixture was extracted with $CHCl_3$ and the dried extract was distilled to give a 77% yield of a product, identical with that prepared by the above procedure and included in Table II.

N-(*p*-Toluenesulfonyl)-N-(2-hydroxyethyl)aniline (XIII).—To 13.7 g (0.1 mole) of 2-anilinoethanol (XII) was added, in one batch with cooling, 20.95 g (0.11 mole) of *p*-toluenesulfonyl chloride. The mixture was stirred for 10–15 min. To the cold mixture was then added dropwise with stirring 53 ml of pyridine. The mixture was allowed to stir for 50–60 min in an ice bath and then poured with vigorous stirring into crushed ice. The thick paste obtained was dissolved in acetone and precipitated with anhydrous ether to give a quantitative yield, mp 71–73°.

Anal. Calcd for $C_{15}H_{17}NO_3S$: C, 61.83; H, 5.88; N, 4.81. Found: C, 62.01; H, 5.80; N, 4.95.

N-(*p*-Toluenesulfonyl)-N-(2-*p*-toluenesulfonyloxyethyl)aniline (XIV).—Use of 41.9 g (0.22 mole) of *p*-toluenesulfonyl chloride in the above sequence gave a solid which was recrystallized from methanol to give the product, mp 120–122°, in 90% yield. Recrystallization from methanol gave material, mp 125–126°.

Anal. Calcd for $C_{22}H_{23}NO_5S_2$: C, 59.30; H, 5.20; N, 3.14; S, 14.39. Found: C, 59.15; H, 5.00; N, 3.12; S, 14.55.

N-(2-Fluoroethyl)-N-(*p*-toluenesulfonyl)aniline (XV).—A mixture of 9.0 g (0.02 mole) of XIV and 5.8 g (0.1 mole) of anhydrous KF in 150 ml of DMF was refluxed for 48 hr. The mixture was filtered, cooled, and poured into cold water to give the product as a solid. The solid was dissolved in cold methanol and dilution with water gave the product, mp 73–74°, in 51% yield.

Anal. Calcd for $C_{15}H_{16}FNO_2S$: C, 61.41; H, 5.49; N, 4.77. Found: C, 61.42; H, 5.68; N, 4.94.

N-(*p*-Toluenesulfonyl)-N-(2-hydroxyethyl)-*o*-toluidine (XVII). Using XVI and an equimolar quantity of *p*-toluenesulfonyl chloride as described in the preparation of XIII, this compound was obtained in quantitative yield. Recrystallization from cyclohexane gave a solid, mp 77–79°.

Anal. Calcd for $C_{16}H_{19}NO_3S$: C, 62.92; H, 6.27; N, 4.59; S, 10.50. Found: C, 62.90; H, 6.32; N, 4.76; S, 10.60.

N-(2-Fluoroethyl)-N-(*p*-toluenesulfonyl)-*o*-toluidine (XIX). Use of XVI and *p*-toluenesulfonyl chloride in a 2:1 molar ratio as described for the preparation of XIV gave a quantitative yield of crude product which was then refluxed in methanol with anhydrous KF to give the product, mp 131–133° (from acetone–ether).

Anal. Calcd for $C_{16}H_{18}FNO_2S$: C, 62.51; H, 5.90; N, 4.56; F, 6.18. Found: C, 62.28; H, 5.65; N, 4.34; F, 5.94.

4-Hydroxy-2-butanone Thiosemicarbazone, a Potential Anticancer Agent

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The high antimycobacterial activity of *p*-acetaminobenzaldehyde thiosemicarbazone reported by Domagk[1] stimulated various workers to prepare numerous thiosemicarbazones as potential antimicrobial,[2,3] antiviral,[4] antifungal,[5] and anticancer agents.[6] For several years we have synthesized and studied the effects of a number of thiosemicarbazone derivatives as potential chemotherapeutic agents. The results have indicated that certain aliphatic thiosemicarbazones may possess anticancer activity *in vivo*.[6] This report describes the synthesis, purification, chemical and physical properties, and tests for acute toxicity of 4-hydroxy-2-butanone thiosemicarbazone as a potential anticancer agent against Lewis lung carcinoma in BDF1 mice. Studies with the compound reported herein have shown that it has an effect against this tumor.

Experimental Section

4-Hydroxy-2-butanone Thiosemicarbazone.—A hot solution of thiosemicarbazide (9.1 g, 0.1 mole) in distilled water (150 ml) was added to a mixture of 8.8 g (0.1 mole) of 4-hydroxy-2-butanone and 5 ml of glacial acetic acid in ethanol (100 ml) and the resulting mixture refluxed for 3 hr. After cooling, the insoluble condensation product was filtered, washed with water and petroleum ether (bp 30–60°), and dried. The product was purified by recrystallizing twice from 70% ethanol to give a 90% yield of shiny white crystals, mp 142–145°.

Anal. Calcd for $C_5H_{11}N_3OS$: C, 37.24; H, 6.88; N, 26.07. Found: C, 36.99; H, 6.80; N, 26.04.

Toxicity and Antitumor Studies. Acute toxicity studies were performed in the BDF1 strain of mice as maintained at the National Institutes of Health, Bethesda, Md., according to a procedure described previously.[6] This strain of mice was also used in the antitumor studies. All of the animals tolerated 500 mg/kg. The compound was tested for antitumor activity against four tumor systems, Sarcoma 180, Dunning ascites leukemia, Leukemia 1210, and Lewis lung carcinoma by screeners under contract to the Cancer Chemotherapy National Service Center. The testing procedures employed have been described previously.[7]

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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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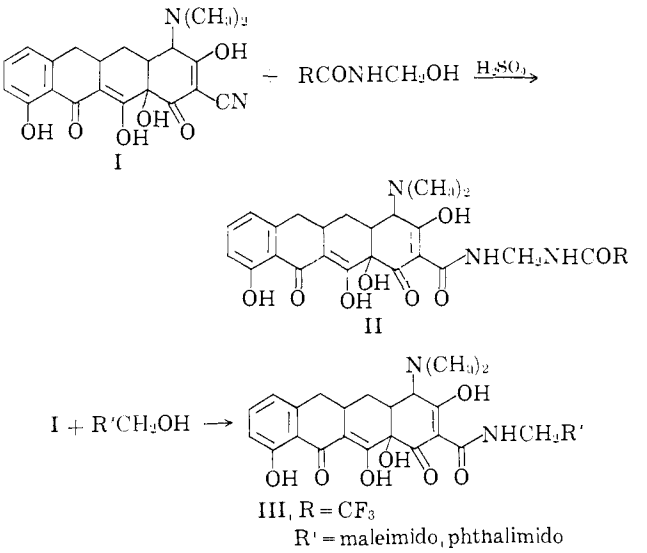
Although the facile conversion of the 2-carboxamido group in the tetracycline series into a nitrile by means of an acid chloride, such as benzenesulfonyl or methanesulfonyl chloride in pyridine has been known for some time,[1] only one reaction has appeared which utilizes the nitrile.[2] In that case 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

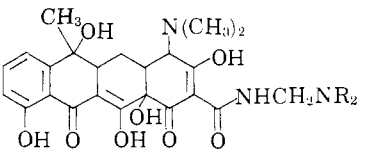


The reactions of these substances with the aromatic ring of tetracyclines are reported by us in an accompanying paper.

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

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

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