The Synthesis and Antitumor Properties of a 6-Alkoxytetrahydrooxazine

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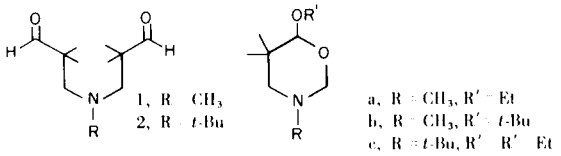
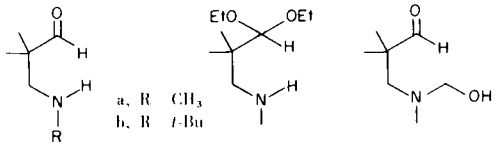
Recently we reported on the antitumor properties of a hindered *N*-*t*-butyl-3,3′-iminodiester (1). Our continuing interest in this area has led us to undertake the synthesis of the corresponding *N*-methyl and *N*-*t*-butyl-3,3′-imino-2,2,2′,2′-tetramethyldipropanals (**1** and **2**).



1,2 **3a,b,c**



4a,b **5** **6**

Attempts to synthesize the less hindered *N*-methyl dialdehyde, **1**, by a modification of the Mannich reaction using two moles of isobutyraldehyde and formaldehyde and one mole of methylamine hydrochloride in ethanol did not give the dialdehyde but rather gave several products including 6-ethoxytetrahydro-3,5,5-trimethyl-2*H*-1,3-oxazine (**3a**) and minor products *N*-methyl-3-imino-2,2-dimethylpropanal (**4a**) and its diethylacetal **5**.

When only one mole of isobutyraldehyde was employed and a benzene azeotrope was used to drive the reaction to completion, **3a** was obtained in 60% yield. When *t*-butyl alcohol was used in place of ethanol, 6-*t*-butoxytetrahydro-3,5,5-trimethyl-2*H*-1,3-oxazine (**3b**) was obtained from a mixture in 25% yield.

While 6-ethoxytetrahydro-3-*t*-butyl-5,5-trimethyl-2*H*-1,3-oxazine (**3c**) could not be made from *t*-butylamine hydrochloride, it was synthesized in 58% yield using *t*-butyl amine in place of its salt.

In view of the medicinal value of many oxazine and related derivatives, and since their activity in many cases is thought to be related to their ability of undergo rapid hydrolysis in aqueous solution (2,3,4), the 6-alkoxy oxazines were screened for their antineoplastic activity. This new class of molecules would be expected to undergo either fast or slow hydrolysis in aqueous solutions depending on the steric factors related to the alkyl group on nitrogen and to the 6-alkoxy group.

The more readily hydrolyzed oxazine, **3a**, was shown to display significant antineoplastic activity against lymphocytic and mild activity against lymphoid leukemia. Since aldehyde **4a**, the final hydrolysis product of **3a**, is not stable, we were unable to analyze its activity, however, acetal **5**, a simple precursor of **4a**, displayed only mild activity against lymphocytic leukemia. Table I summarizes the pertinent screening results for compounds **3a** and **5**. Because of the low activity of **5** as shown in Table I, we feel **4** is not an important intermediate. Therefore, synthetic studies are under way to show that a possible intermediate hydrolysis product of **3a**, perhaps **6**, could be a metabolite of **3a** and possibly account for its antineoplastic activity. We have been unable to isolate such an intermediate to date. The aldehyde group of **6** is capable of forming an imine linkage with RNA or DNA bases and the $R_2N\text{-}CH_2OR'$ group (R′ = H, SO_3H, PO_3H, or glucose) is a potential alkylating agent in its iminonium ion form ($R_2N^+{=}CH_2$) and could act as a second alkylating agent relating these molecules to the well known nitrogen mustards (5).

TABLE I

in vivo Data for **3a** and **5**

Compound	Tumor	Dose (mg./kg.)	Activity (% T/c) (c,d)
3a	LE (a)	200	126
	PS (b)	200	140
		150	154
		100	150, 140
		66	136
		44	154
5	PS	400	131

(a) LE = L-1210 lymphoid leukemia. (b) PS = P388 lymphocytic leukemia. (c) Ratio of survival time of treated animals (mice) to control animals expressed as %. (d) Testing was kindly performed by CCNSC, National Cancer Institute (N.I.H.).

EXPERIMENTAL

Preparation of **3a**, **b** and **c**.

In a typical preparation, given here for **3a**, methylamine hydrochloride, 16.9 g. (0.25 mole), formaldehyde (trioxane), 15 g. (0.5 mole), and 0.5 ml. of concentrated hydrochloric acid were refluxed under nitrogen in 200 ml. of ethanol for 1 hour at which time isobutyraldehyde, 18 g. (0.25 mole) was added dropwise to the mixture. After an additional 3 hours reflux period, 200 ml. of benzene was added to the mixture and 100 ml. of solvent was removed as an azeotrope. The reaction was cooled to 0° and 100 ml. of ice water was added to it. The aqueous layer was washed with ether and made basic with sodium carbonate. The basic layer was extracted with ether which was dried with potassium carbonate, filtered, and evaporated to give an oil which was distilled to give 27 g. (60%) of **3a**; b.p. 42-45°/0.2 mm; **3b**, b.p. 49-51°/0.5 mm. The ir, nmr, and mass spectra data were consistent with structure assigned. We were unable to obtain proper analysis for these labile molecules, however, they were readily hydrolyzed in acid to aldehyde **4a** which was characterized as its benzenesulfonamide, m.p. 54-55°.

Anal. Calcd. for $C_{12}H_{17}NO_3S$: C, 56.49; H, 6.66; N, 5.48. Found: C, 56.20; H, 6.48; N, 5.23.

Tetrahydrooxazine **3c** was made in the same manner using *t*-butylamine, except no acid catalysis was used. The product was obtained by distillation of the reaction mixture after the azeotrope step (b.p. 43-45°/0.5 mm) and characterized as the 2,4-DNP-H_2SO_4 salt of aldehyde **4b**, m.p. 205-205.5°.

Anal. Calcd. for $C_{15}H_{24}N_5O_8S$: C, 41.56; H, 5.57; N, 16.10. Found: C, 41.48; H, 5.91; N, 16.02.

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