Å., owing to the variation possible in conformational preference. The carbonyl oxygen distance from the center of the triple bond ranges from 3.1–4.2 Å.

An allowed pattern of atoms can thus be drawn for oxotremorine, Fig. 5. This calculated energy-allowed pattern clearly mirrors the muscarinic pattern, previously proposed, Fig. 6.

It can be concluded that oxotremorine can assume a muscarinic pharmacophore, within its structure, on the basis of theoretical calculations of its preferred conformations. Although conclusive pharmacological evidence that would brand oxotremorine as a central muscarinic agonist is still lacking, these studies implicate the feasibility of this mechanism.

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Antiradiation Compounds XIII: 1-(Dithioacetic Acid)-Pyridinium Betaines

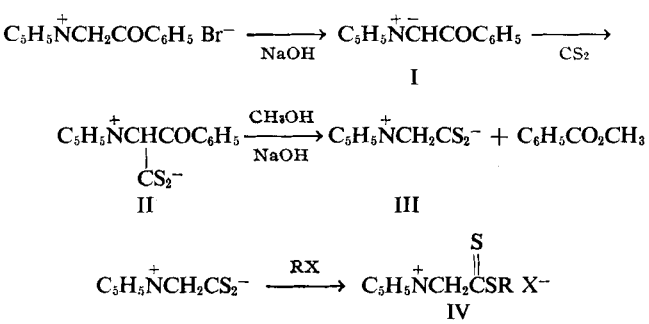
WILLIAM O. FOYE, YOUNG JA CHO, and KYUNG HEE OH

Abstract □ Active mono-*S*-alkyl esters prepared from 1-(dithioacetic acid)-pyridinium betaine (III) were found to be sufficiently stable for screening as radiation-protective agents, and *e*-withdrawing substituents in the pyridine ring gave stable betaines. Reaction of the methyl ester of III with phenacyl bromide and alkali resulted in *S*-alkylation to give a ketene mercaptal betaine (VIII). Both the allyl and *p*-nitrobenzyl esters of 1-(dithioacetic acid)-pyridinium halides were radiation-protective in mice, and betaines with substituents in the pyridine ring were radiation-protective in a bacterial test.

Keyphrases □ Antiradiation compounds—synthesis □ 1-(Dithioacetic acid)-pyridinium betaines—synthesis □ Pharmacological screening—antiradiation compounds □ IR spectrophotometry—structure

Amino and guanidino zwitterions containing the thiosulfate (1), phosphorothioate (2), and trithiocarbonate (3) groups, as well as other zwitterionic structures (4) which contain the β-mercaptoethylamine moiety have shown appreciable radiation-protective abilities in mice. α-Acetamidinium thiosulfate zwitterions (5) have also shown good radiation-protective properties. It appeared likely, therefore, that other zwitterions containing, or giving rise to, a thiolate anion should be radiation-protective. Dithioacetic acid pyridinium betaines, obtained from the reaction of carbon disulfide and pyridinium ylids, appeared to have the necessary structural requirements for protective activity in a charged nitrogen and a thiolate anion, and were therefore investigated for possible radiation-protective properties.

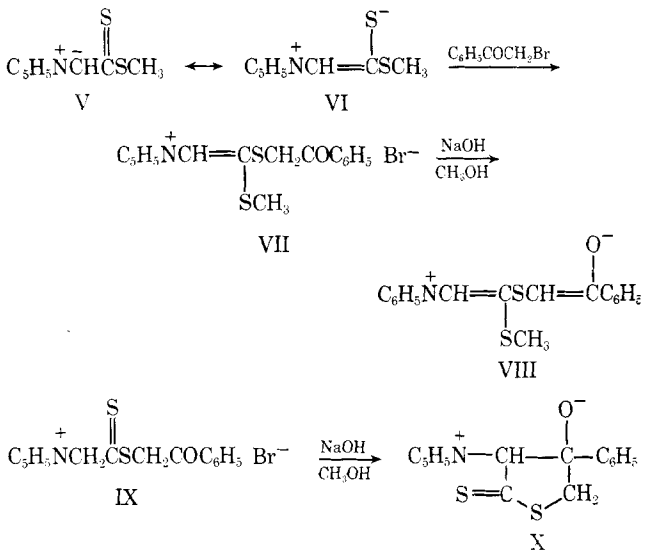
Pyridinium betaines are compounds, termed by Kröhnke (6), which contain a negatively charged carbon, oxygen, or sulfur adjacent to, or at greater distance from, the positively charged nitrogen; the term ylid is now generally used where the charges are on adjacent atoms. When phenacylpyridinium bromide is treated with alkali, a red solution of the ylid (I) results. Reaction with carbon disulfide gives the α-dithiocarboxylate zwitterion (II) which decomposes to the dithioacetic acid betaine (III) in methanolic alkali (6). This compound, although unstable, can be isolated, and remains stable long enough for derivatives to be prepared. Conversion to the active allyl and *p*-nitrobenzyl esters (IV) was carried out, and compounds of sufficient stability for antiradiation screening were obtained.



Reaction of the dithioacetic acid betaine (III) with bromoethylamine hydrobromide did not give the desired ester; also, no reaction with phenyl halides or

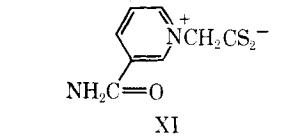
2,4-dinitrochlorobenzene took place. Kröhnke *et al.* (7) have found that the methyl ester of dithioacetic acid pyridinium betaine exists in methanolic alkali as both the carbon ylid (V) and the sulfur ylid (VI), and that alkyl halides react preferentially with the thiolate anion. To determine whether a di-*S*-alkyl derivative would form preferentially to an *S*-alkyl-C-alkyl derivative, reaction of the methyl ester (IV) with phenacyl bromide was carried out. The product, according to the IR spectrum, was the ketene mercaptal, 2-methylthio-2-phenacylthio-1-vinylpyridinium bromide (VII). In the IR, both C=O and C=C absorption were found; no absorption due to C=C would be expected in the C-alkylation product of IV.

Treatment of VII with methanolic alkali gave yellow crystals of a low-melting, unstable compound from which HBr had been lost. This compound still revealed IR absorption due to both C=O and C=C, as well as enol, and was therefore concluded to be the betaine (VIII). Similar treatment of the phenacyl ester of 1-(dithioacetic acid)-pyridinium bromide (IX) by Kröhnke (6) led to ring-closure to give a stable, high-melting dihydrothiophene betaine (X), which was ultimately converted to a thiophene. Compound X was prepared, and its IR spectrum showed no evidence of either C=O or C=C. Similar attempts to convert VIII to a dihydrothiophene resulted in decomposition.

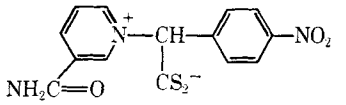


Besides making modifications in the dithioacetic acid portion of the molecule, the authors also desired to learn the effects that *e*-withdrawing substituents in the pyridine ring might have both on stability of the betaines and their radiation-protective ability. Accordingly, 1-phenacyl and 1-*p*-nitrobenzyl derivatives of nicotinamide, ethyl nicotinate, and ethyl isonicotinate were prepared. Reaction of 1-phenacyl-3-carbamylpyridinium bromide with carbon disulfide and alkali gave at once a red solution from which the dithioacetic acid betaine (XI) was isolated. The compound could not be recrystallized without decomposition, but it was stable after being dried.

Treatment of 1-*p*-nitrobenzyl-3-carbamylpyridinium chloride with carbon disulfide in alkali gave a red solution only slowly. Isolation of the product and elemental analysis showed that the *p*-nitrophenyl group had not been removed; the α-dithiocarboxylic acid betaine XII was obtained.



XI



XII

Similarly, reaction of both 1-phenacyl- and 1-*p*-nitrobenzyl-3-carbethoxypyridinium halides with carbon disulfide and alkali gave the α-dithiocarboxylic acid betaines without loss of the benzoyl or *p*-nitrophenyl groups. The same result was obtained with the 1-phenacyl- and 1-*p*-nitrobenzyl-4-carbethoxypyridinium halides; in these cases, very poor analyses were obtained for the products. None of these compounds could be recrystallized without decomposition, but they were relatively stable after drying.

RADIATION-PROTECTIVE PROPERTIES[1]

Tests were carried out in mice *versus* 825r (X-rays) with an observation period of 30 days. The *p*-nitrobenzyl ester (IV) of dithioacetic acid pyridinium chloride gave fair protection (33%), and the allyl ester (IV) provided slight protection (17%) to mice. In antiradiation testing on bacteria, 1-(dithioacetic acid)-3-carbamylpyridinium betaine (XI) gave good protection (>45%), and the α-*p*-nitrophenyl-3-carbamyl (XII), α-benzoyl-3-carbethoxy (XIII), and α-*p*-nitrophenyl-4-carbethoxy pyridinium dithioacetic acid betaines gave poor protection (1–24%).

EXPERIMENTAL

Analyses for carbon, hydrogen, and nitrogen were done by Weiler and Strauss, Oxford, England. Sulfur analyses were done by Parr bomb peroxide fusion. Melting points were taken on a Mel-Temp apparatus and are corrected. IR absorption spectra were obtained with a Perkin-Elmer model 137B spectrometer.

1-(Dithioacetic acid)-pyridinium Betaine (III)—Phenacylpyridinium bromide (6) (28 g., 0.10 mole) was dissolved in 120 ml. of methanol, and 8 ml. of carbon disulfide (0.12 mole) was added at room temperature, followed by 60 ml. of 2 *N* methanolic sodium hydroxide. After 2 hr., the orange crystals were filtered and washed with water. Recrystallization was accomplished from water not over 60°, giving 15.3 g. (90%), m.p. 110–112° (lit. m.p. 110°) (6). The product decomposed after standing in a desiccator for a day.

Anal.—Calcd. for $C_7H_7NS_2$: S, 37.87. Found: S, 38.05.

1-(Dithioacetic acid p-nitrobenzyl ester)-pyridinium Chloride (IV)—*p*-Nitrobenzyl chloride (1.71 g., 0.01 mole) was dissolved in dimethylformamide (20 ml.), and 1-(dithioacetic acid)-pyridinium betaine (1.57 g., 0.009 mole) was added gradually with stirring. A red solution resulted from which yellow crystals were separated and washed with ether. The yield was 1.03 g. (37%); m.p. 167–168°.

Anal.—Calcd. for $C_{14}H_{13}ClN_2O_2S_2$: C, 49.34; H, 3.80; N, 8.25; S, 18.82. Found: C, 48.66; H, 3.89; N, 7.99; S, 19.25.

1-(Dithioacetic acid allyl ester)-pyridinium Bromide (IV)—1-(Dithioacetic acid)-pyridinium betaine (0.85 g., 0.005 mole) was added to a solution of chloroform (5 ml.) and allyl bromide (2 ml., 0.01 mole). Warming on a water bath gave a red solution, and addition of ether

[1] Antiradiation screening of some of the compounds described has been carried out at the Walter Reed Army Institute of Research, and results have been reported through the courtesy of Drs. D. P. Jacobus and T. R. Sweeney.

produced yellow crystals, which were filtered and washed with ether. The yield was 1.0 g. (71%); m.p. 107–111°.

Anal.—Calcd. for $C_{10}H_{12}BrNS_2$: C, 41.38; H, 4.40; N, 4.82; S, 22.10. Found: C, 40.92; H, 4.46; N, 4.67; S, 22.68.

1 - (2 - Methylthio - 2 - phenacylthio - 1 - vinyl) - pyridinium Bromide (VII)—1-(Dithioacetic acid methyl ester)-pyridinium betaine (6) (1.83 g., 0.01 mole) was dissolved in 5 ml. of chloroform, and phenacyl bromide (2.0 g., 0.01 mole) was added. A yellow solution resulted, and addition of ether and refrigeration for a few days produced yellow crystals; 3.08 g. (81%), m.p. 90–95°; IR (KBr) 1675 (C=O), 1630 (C=C), 1600 (doublet, ring stretch) cm.$^{-1}$.

Anal.—Calcd. for $C_{16}H_{16}BrNOS_2$: C, 50.26; H, 4.19; N, 3.66; S, 16.77. Found: C, 49.71; H, 4.26; N, 3.58; S, 16.20.

1 - (2 - Methylthio - 2 - phenacylthio - 1 - vinyl) - pyridinium Betaine (VIII)—To a solution of the previous product (3.82 g., 0.01 mole) in 5 ml. of methanol cooled by an ice bath was added 2 *N* methanolic sodium hydroxide. After the solution was refrigerated for 1 hr., yellow crystals appeared and were filtered and washed with water. Recrystallization from methanol gave 2.35 g. (75%), m.p. 80–83°; IR (mineral oil) 1675 (C=O), 1615 (C=C), 1575 (enol) cm.$^{-1}$.

Anal.—Calcd. for $C_{16}H_{15}NOS_2$: C, 63.78; H, 4.19; N, 4.64; S, 21.28. Found: C, 63.32; H, 4.67; N, 4.24; S, 21.03.

1-Phenacyl-3-carbethoxypyridinium Bromide—A solution of ethyl nicotinate (8), (6.53 g., 0.044 mole), phenacyl bromide (7.86 g., 0.044 mole), and methanol (60 ml.) was refluxed for 16 hr. The solvent was removed by a stream of air, and the residue was washed with ether. Recrystallization from ether–methanol gave 14 g. (93%); m.p. 181–182°.

Anal.—Calcd. for $C_{16}H_{16}BrNO_3$: C, 54.87; H, 4.61; N, 4.00. Found: C, 54.61; H, 4.65; N, 3.62.

1-(p-Nitrobenzyl)-3- carbethoxypyridinium Chloride—A solution of ethyl nicotinate (8) (3.02 g., 0.02 mole), *p*-nitrobenzyl chloride (3.24 g., 0.02 mole), and methanol (30 ml.) was refluxed for 35 hr. Addition of ether produced yellow crystals which were collected and recrystallized from ether–methanol, giving 2.15 g. (41%); m.p. 96–97°.

Anal.—Calcd. for $C_{15}H_{15}ClN_2O_4$: C, 55.82; H, 4.68; N, 8.68. Found: C, 55.66; H, 4.95; N, 8.85. . .

1-Phenacyl-4-carbethoxypyridinium Bromide—A solution of ethyl isonicotinate (8) (7.55 g., 0.05 mole), phenacyl bromide (9.95 g., 0.05 mole), and methanol (95 ml.) was refluxed for 5 hr. The solvent was evaporated, and the residue was washed with ether. The product was recrystallized from methanol, giving 15.1 g. (85%) of yellow crystals, m.p. 183.5–185°.

Anal.—Calcd. for $C_{16}H_{16}BrNO_3$: C, 54.87; H, 4.61; N, 4.00. Found: C, 54.60; H, 4.51; N, 4.12.

1-(p-Nitrobenzyl)-4-carbethoxypyridnium Chloride—A solution of ethyl isonicotinate (8) (7.55 g., 0.05 mole), *p*-nitrobenzyl chloride (8.56 g., 0.05 mole), and methanol (25 ml.) was refluxed for 24 hr. The solvent was removed by a stream of air, and the residue was washed with ether. Recrystallization from ether–methanol gave 8.0 g. (49%); m.p. 173.5–174°.

Anal.—Calcd. for $C_{15}H_{15}ClN_2O_4$: C, 55.82; H, 4.68; N, 8.68. Found: C, 55.32; H, 4.75; N, 8.82.

1-(Dithioacetic acid)-3-carbamylpyridinium Betaine (XI)—1-Phenacyl-3-carbamylpyridinium bromide (9) (11 g., 0.034 mole) dissolved in 1 l. of methanol was treated with carbon disulfide (2.6 g., 0.34 mole) dissolved in 4 ml. of methanol, and the solution was stirred at room temperature for 25 min. It was then treated with 20 ml. of 2 *N* NaOH in methanol, stirred at 5–10° for 25 min., and stored over dry ice overnight. The orange precipitate was filtered, washed with cold ether, and dried *in vacuo*. The yield was 2.53 g. (35%); m.p. 104.5–107°.

Anal.—Calcd. for $C_8H_8N_2OS_2$: C, 45.26; H, 3.79; N, 13.19. Found: C, 45.36; H, 3.92; N, 12.95.

1-[α-(p-Nitrophenyl)dithioacetic acid]-3-carbamylpyridinium Betaine (XII)—1-(*p*-Nitrobenzyl)-3-carbamylpyridinium chloride (9) (2.93 g., 0.01 mole) dissolved in 250 ml. of methanol was treated with carbon disulfide (1 g., 0.013 mole) dissolved in 5 ml. of methanol. Addition of 6 ml. of 2 *N* NaOH in methanol gave a red solution which deposited greenish crystals when stored over dry ice. A yield of 2.19 g. (67%) was obtained; m.p. 115–117°.

Anal.—Calcd. for $C_{14}H_{11}N_3O_3S_2$: C, 50.43; H, 3.32; N, 12.63. Found: C, 49.97; H, 3.44; N, 12.39.

1-(α-Benzoyldithioacetic acid)-3-carbethoxypyridinium Betaine (XIII)—To 1-phenacyl-3-carbethoxypyridinium bromide (3.5 g., 0.01 mole) in 32 ml. of methanol was added carbon disulfide (1 g., 0.013 mole) in 5 ml. of methanol, and the solution was stirred for 15 min. Addition of 6 ml. of 2 *N* NaOH in methanol gave a red solution from which a black precipitate appeared after several hours at room temperature. The product was collected and washed with cold ether, giving 0.77 g. (29%) of red-black solid; m.p. 120–124°.

Anal.—Calcd. for $C_{17}H_{15}NO_3S_2$: C, 59.11; H, 4.37; N, 4.06. Found: C, 59.84; H, 3.99; N, 4.23.

1-[α-(p-Nitrophenyl)dithioacetic acid]-3-carbethoxypyridinium Betaine—1-*p*-Nitrobenzyl-3-carbethoxypyridinium chloride (2 g., 0.006 mole) in 10 ml. of methanol was treated with carbon disulfide (0.5 g., 0.006 mole) in 2 ml. of methanol, and the solution was refluxed 3 hr. After addition of 2 *N* NaOH in methanol (3.6 ml.), a red precipitate appeared, which was filtered and dried. The yield was 1.7 g. (75%); m.p. 125–126°.

Anal.—Calcd. for $C_{16}H_{14}N_2O_4S_2$: C, 53.02; H, 3.95; N, 7.74. Found: C, 53.17; H, 4.53; N, 8.37.

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