N-(3-Phthalidyl)-tetracycline, a New Carboxamido Derivative of Tetracycline

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Received September 24, 1962

Condensation reactions involving the carboxamido group of tetracycline have been reported by Gottstein, *et al.*[1] These workers describe an xanthydrol condensate of tetracycline as well as numerous derivatives prepared from amines and formaldehyde by the Einhorn reaction. One of these, N-(pyrrolidinomethyl)-tetracycline,[2] has proved superior to tetracycline when administered parenterally.[3,4]

The reactivity of the carboxamido moiety of tetracycline, and the interesting properties of its condensation products, has prompted us to evaluate the reaction of this amide with phthalaldehydic acid. Wheeler, *et al.*,[5] have shown that phthalaldehydic acid reacts with amines, amides, urethanes, alcohols and phenols giving rise to 3-substituted phthalides. It is believed that this acid reacts with the carboxamido group of tetracycline giving N-(3-phthalidyl)-tetracycline (I) as the reaction product. As noted with N-(9-xanthyl)-tetracycline,[6] I also failed to yield an Einhorn or Mannich derivative when it reacted with formaldehyde and morpholine or pyrrolidine. This is consistent with the proposed amide substitution for I. It also provides further evidence that the Einhorn rather than Mannich reaction normally occurs in aminoalkylations of tetracycline.

Using a turbidometric assay against *Micrococcus pyogenes var. aureus*, I was found to retain antibacterial activity equivalent to that contributed by the tetracycline portion of the molecule. It is soluble in water at pH 9 or greater but is virtually insoluble in 0.1 to 6.0 *N* hydrochloric acid. Unlike N-(pyrrolidinomethyl)-tetracycline, I gave no significant blood levels orally in dogs or intramuscularly in rabbits.

Experimental

Fifty grams (0.1 mole) of tetracycline base hydrate was slurried in 500 ml. of water and heated to 90 to 95°. This temperature was maintained throughout the course of the reaction. Phthalaldehydic acid (72 g., 0.48 mole) then was added rapidly with moderate agitation forming a yellow solution. In 5 min. a copious quantity of gum separated. After 15 min., heat and stirring were discontinued and the reaction mixture was allowed to cool to 25° for 2 hr. The gum was isolated by decantation and washed with 100 ml. of water. It then was dissolved in acetone at 50° from which it crystallized immediately. The crystals were removed by filtration, recrystallized from acetone and dried overnight at 90° under vacuum. A yield of 40 g. (71.3%) was obtained. I also was prepared by heating an equimolar mixture of the reactants at 120° for 0.5 hr. and crystallizing from acetone.

Anal. Calcd. for $C_{30}H_{25}N_2O_{10}$: C, 62.5; H, 4.90; N, 4.86. Found: C, 62.18, 62.5; H, 4.89, 5.03; N, 4.79, 4.9.

The compound had m.p. 191–193° dec., (capillary); $[\alpha]^{25}D$ −157° (*c* 1 in 0.1 *N* HCl in methanol); absorption maxima in 0.1 *N* HCl in methanol at 222 mμ (ϵ = 23,865), 272 mμ (ϵ = 20,867), 362 mμ (ϵ = 15,103); infrared absorption maxima (KBr pellet) 2.95, 3.25, 3.5, 5.6, 6.0, 6.2, 6.3 and 6.4 μ.

Acknowledgments.—The authors are indebted to Messrs. Richard Downing, James Lannon, John Messerly and David Whitehead for determination of the biological, chemical and physical data.

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Compounds Related to Carnitine: Derivatives of 4-Dimethylamino-3-hydroxybutyric Acid

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Received August 28, 1962

Carnitine (I) is an ubiquitous substance first extracted in 1905 from mammalian muscle tissue,[1] however, its structure was not fully elucidated until 1927 when Tomita and Sendju[2] succeeded in resolving synthetic *dl*-carnitine and showed that the *l*-isomer was identical with the naturally occurring material. Vitamin B_T, an essential factor to the mealworm (*Tenebrio molitor*), has been shown to be identical with carnitine.[3] The substance affects the oxidation of long chain fatty acids in liver homogenates[4] and its coenzyme A ester has been isolated from brain tissue and is reported to have an inhibitory action on neural transmission.[5] Recently[6] 4-dimethylamino-3-hydroxybutyric acid (II) and carnitine (I) have been tentatively identified as metabolites of 4-dimethylaminobutyric acid in the rat. Positive identification was not possible in the work cited, however, since a synthetic sample of II was lacking. As part of a study conducted in these laboratories designed partially to elucidate the possible function of carnitine and its derivatives in affecting the central nervous system and their part in mammalian lipid metabolism it became necessary to prepare II and several of its analogs, namely ethyl 4-dimethylamino-3-hydroxybutyrate (III), 4-dimethylaminobutane-1,3-diol (IV) and 4-dimethylamino-3-hydroxybutyramide (V).

$(CH_3)_3N^+CH_2CHOHCH_2COO^-$
I

$(CH_3)_2NCH_2CHOHCH_2Y$
II, Y = COOH
III, Y = $COOC_2H_5$
IV, Y = CH_2OH
V, Y = $CONH_2$

Compounds II, IV and V have been compared to carnitine in their ability to increase the oxidation of palmitate by incubated heart muscle particulates, and it was found that II was nearly as active as carnitine while IV and V were inactive by this test.[7] None of these

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compounds have exhibited significant CNS activity in the course of preliminary screening.

Experimental[8]

4-Dimethylamino-3-hydroxybutyric Acid (II).—4-Amino-3-hydroxybutyric acid[9] (3 g.), water (150 ml.), 37% formaldehyde (4.08 g.) and 10% palladium on carbon catalyst (4 g.) were placed in a pressure bottle and hydrogenated on a Parr apparatus, using an initial pressure of 2.15 kg./cm.^{2}. Uptake of hydrogen was complete within 1 hr. and the shaking was continued for an additional 0.5 hr. with no additional change in pressure. The mixture was filtered free of catalyst (Celite) and the filter cake was washed with hot water. The combined filtrate and wash was evaporated on a steam bath *in vacuo* to give an oil which crystallized as white, flat needles when treated with ethyl acetate (3 g., 80%); m.p. 145–147°. For analysis a portion of this material was recrystallized 3 times by dissolving in a minimum of methanol, adding ethyl acetate and removing the methanol as an azeotrope. The melting point was unaffected by this treatment.

Anal. Calcd. for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52; NCH_3, 20.43. Found: C, 48.67; H, 8.84; N, 9.43; NCH_3, 18.4.

Ethyl 4-Dimethylamino-3-hydroxybutyrate (III).—Anhydrous hydrogen chloride was bubbled for 2 hr. into a suspension of 4-dimethylamino-3-hydroxybutyric acid (558.5 g., 3.80 moles) in absolute ethanol (5.7 l.) containing ethyl orthoformate (283.8 g., 1.95 moles) while protecting the reaction mixture with a drying tube. The suspended material dissolved during this time yielding a clear yellow solution which was heated under reflux for 2 hr. and then concentrated to a small volume *in vacuo*. The syrupy residue was diluted with ice water and made basic with sodium carbonate. The basic solution was extracted 5 times with chloroform. The combined chloroform extracts were washed with a small volume of water and dried over anhydrous sodium sulfate. The dried chloroform solution was concentrated and the residue was distilled to yield the ethyl ester (532 g., 80%); b.p. 90–93° (4–5 mm.). The substance behaved as a pure compound when subjected to paper chromatography and electrophoresis.

Anal. Calcd. for $C_8H_{17}NO_3$: C, 54.83; H, 9.78; N, 7.99; eq. wt., 175.22. Found: C, 54.72; H, 9.88; N, 8.23; eq. wt., 179.7.

4-Dimethylaminobutane-1,3-diol (IV).—Ethyl 4-dimethylamino-3-hydroxybutyrate (12 g., 0.07 mole) was added slowly with stirring to lithium aluminum hydride (3.5 g.) in tetrahydrofuran (75 ml.). At the conclusion of the addition the mixture was heated to reflux and stirred for 45 min. It then was allowed to stand overnight at room temperature and decomposed by the cautious addition of water (6.5 ml.). The mixture was filtered free of alumina (Celite) and the filter cake was washed with several portions of tetrahydrofuran. The combined filtrate and wash was concentrated to remove solvent and the residue was distilled *in vacuo*. The alcohol was obtained as a main fraction, b.p. 103–104° (6 mm.) (85%), with very little forerun, (n^{24}D 1.4574). The infrared spectrum showed the absence of carbonyl absorption.

Anal. Calcd. for $C_6H_{15}NO_2$: C, 54.10; H, 11.35; N, 10.52. Found: C, 54.23; H, 11.34; N, 10.65.

4-Dimethylamino-3-hydroxybutyramide (V).—Ethyl 4-dimethylamino-3-hydroxybutyrate (48 g.) and liquid ammonia (450 ml.) were placed in a stainless steel bomb and autoclaved overnight at 150°. The bomb was cooled to room temperature and the excess ammonia was allowed to distil off leaving an oily residue which was taken up in chloroform and applied to a column of Merck acid washed alumina (15 × 4.35 cm.). The product was eluted with the solvent front and yielded crystals (25 g.) from a mixture of chloroform and Skellysolve C, m.p. 81–83°. A small portion of this material was recrystallized 3 times from the same solvent mixture to give crystals m.p. 85–86°.

Anal. Calcd. for $C_6H_{14}N_2O_2$: C, 49.30; H, 9.65; N, 19.17. Found: C, 49.21; H, 9.52; N, 18.99.

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Acknowledgment.—We wish to thank Miss T. Rebane and Mr. J. Bunker for their competent technical assistance.

Synthesis of Isothiocyanates as Potential Antineoplastic Agents[1,2]

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Received August 22, 1962

The hypothesis that known alkylating agents react *in vivo* with the nucleophilic centers encountered in the biological system (amino groups, sulfhydryl groups, organic and inorganic anions)[4] has encouraged chemists to synthesize a large number of compounds which have a high reactivity toward the above mentioned nucleophilic centers as antineoplastic agents. Hendry and colleagues,[5] and later Ross[4] studied some acid chlorides, anhydrides, isocyanates and isothiocyanates (compounds reactive toward amino and alcoholic groups) as antineoplastic agents. Most of these agents were simple organic compounds, *e.g.*, 1,5-diisothiocyanatonaphthalene and 2,4,6-triisothiocyanatotoluene. Later, Bergel and Stock[6] attempted to replace the dichloroethylamino grouping in DL-phenylalanine nitrogen mustard by an isothiocyanate group, in the search of a more selective tumor inhibiting compound. These workers were unable to demonstrate any antitumor effects with such compounds. However, it will be noted from the structures that solubilizing groups are absent. The lack of biological activity might be attributed to a lack of their solubility in the system.

Interest in diisothiocyanates as possible cross-linking agents arose in this Laboratory during the development of fluorescent isothiocyanates as diagnostic agents.[7] It was proposed that solubilizing or conductophoric groupings, *i.e.*, basic side chains, be attached to such molecules. It was expected that basic side chains might transport these compounds throughout the blood stream and to desired sites of action, such as the liver, as has been noted with antimalarial agents. Two isothiocyanates and two diisothiocyanates were synthesized (II, V, VIII and X). It will be noted that X possesses two types of reactive groupings, the isothiocyanato and dichloroethylamino groups.

In this Laboratory, the synthesis of certain purine and pyridine nitrogen mustard compounds has been carried out already, and it was encouraging to note that a purine nitrogen mustard has shown promising results in leukemia.[8] It was proposed, therefore, to

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