NOTES

5-β-Aminoethyltetrazole

By C. Ainsworth

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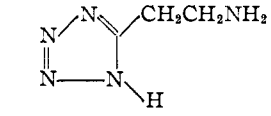
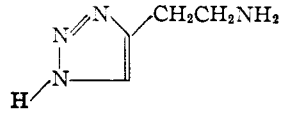
In connection with the investigations of compounds structurally related to histamine,[1] 5-β-aminoethyltetrazole (I) has been synthesized and examined for pharmacological activity.[2]

4-β-Aminoethyl-1,2,3-triazole (II)[3] has only moderate histamine-like activity on isolated smooth muscle strips and on blood pressure, whereas, the isomeric 3-β-aminoethyl-1,2,4-triazole (III)[4] is relatively highly active in these tests. The tetrazole analog, I, therefore, was of special interest in that it represented a replacement by nitrogen of two of the ring carbon atoms of histamine (IV) and could be considered as an analog of each of the compounds, II, III and IV.



I



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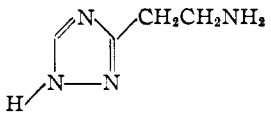
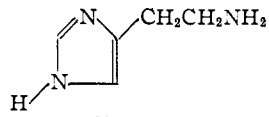
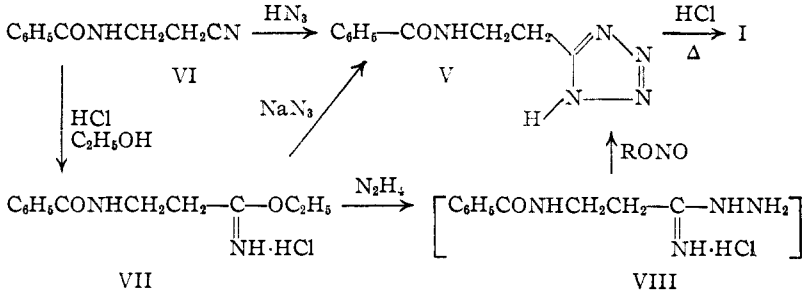


III



IV

Compound I was prepared from 5-β-benzamidoethyltetrazole (V) which, in turn, was obtained from β-benzamidopropionitrile (VI) by three different methods, as indicated in the accompanying reaction scheme



VII VIII

The yields of V were low (about 10%) by each method. Hydrolysis of V with dilute hydrochloric acid gave I which was isolated as the hydrochloride.

Preliminary pharmacological tests have indicated that I, even at high doses, has no histamine-like activity on isolated smooth muscle tissue and on blood pressure.

Acknowledgments.—The author is grateful to H. M. Lee and J. H. Tilden for the pharmacological data; to H. E. Boaz, D. O. Woolf and J. W. Forbes for physical measurements; and to W. L. Brown, H. L. Hunter and G. M. Maciak for the microanalyses.

Experimental[5]

Ethyl β-Benzamidopropionimidate Hydrochloride[6] (VII). —This compound was prepared in quantitative yield by the general procedure for obtaining iminoesters of McElvain and Nelson.[7]

A solution, formed from 8.7 g. (0.05 mole) of β-benzamidopropionitrile,[8] 3 ml. (0.05 mole) of ethanol, 1.8 g. (0.05 mole) of hydrogen chloride and 25 ml. of dioxane, was allowed to stand at 5° for three days. The heavy precipitate of ethyl β-benzamidopropionimidate hydrochloride which formed melted at 120–122°.

Anal. Calcd. for $C_{12}H_{16}N_2O_2 \cdot HCl$: N, 10.92. Found: N, 10.76.

5-β-Benzamidoethyltetrazole (V). (a) Reaction of Ethyl β-Benzamidopropionimidate Hydrochloride and Hydrazine followed by Amyl Nitrite.[9]—To 3.2 g. (0.1 mole) of water-free hydrazine in 50 ml. of ethanol was added, portionwise, with stirring, 25.6 g. (0.1 mole) of ethyl β-benzamidopropionimidate hydrochloride. During the addition the temperature was maintained between −20 and −5° by means of a methanol–Dry Ice-bath. After one hour approximately 15 g. of solid [presumably the hydrazidine hydrochloride (VIII)] was collected on a buchner funnel. The solid was dissolved in 100 ml. of ethanol, the solution cooled to −10° and treated with 10 ml. (0.1 mole) of amyl nitrite. It became pink then red in color, and the temperature of the reaction mixture rose rapidly to 40°. The product (3.5 g.), which separated after standing at 5° for three days, was collected by filtration. After recrystallization from water, 2.2 g. (10%) of 5-β-benzamidoethyltetrazole was obtained as white irregular prisms; m.p. 206°; pK'_a 6.15 (66% dimethylformamide); λ_{max} 224 mμ, log ε 4.06 (methanol).

Anal. Calcd. for $C_{10}H_{11}N_5O$: C, 55.29; H, 5.10. Found: C, 55.23; H, 5.25.

5-β-Benzamidoethyltetrazole was soluble in 1 *N* sodium hydroxide and was reprecipitated upon the addition of acid. It formed a water-insoluble silver salt.

(b) Reaction of Ethyl β-Benzamidopropionimidate Hydrochloride and Sodium Azide.—A mixture of 2.5 g. (0.01 mole) of ethyl β-benzamidopropionimidate hydrochloride, 1 g. (0.015 mole) of sodium azide and 25 ml. of acetic acid was heated under reflux for 24 hours. Sodium chloride (0.4 g.) was removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was dissolved in a minimum amount of hot water and, after cooling, 0.21 g. (10%) of 5-β-benzamidoethyltetrazole separated. The identity with the product obtained by procedure (a) was shown by mixed melting point and infrared spectra.

(c) Condensation of β-Benzamidopropionitrile and Hydrogen Azide.[10]—A water-free solution of hydrogen azide (from 25 g. of sodium azide) in xylene was prepared according to the procedure of Herbst.[11] To this was added 10 g.

(1) (a) H. M. Lee and R. G. Jones, *J. Pharmacol. Exptl. Therap.*, **95**, 71 (1949); (b) R. G. Jones and M. J. Mann, This Journal, **75**, 4048 (1953).

(2) This problem was suggested by Dr. Reuben G. Jones.

(3) J. C. Sheehan and C. A. Robinson, This Journal, **71**, 1436 (1949).

(4) C. Ainsworth and R. G. Jones, *ibid.*, **75**, 4915 (1953).

(5) Melting points were taken on a Fisher–Johns block.

(6) Reported without physical data by A. A. Goldberg and W. Kelly, British Patent 605,952 [*C. A.*, **43**, 672 (1949)].

(7) S. M. McElvain and J. W. Nelson, This Journal, **64**, 1825 (1942).

(8) A. A. Goldberg and W. Kelly, *J. Chem. Soc.*, 1369 (1947).

(9) W. Oberhummer, *Monatsh.*, **63**, 285 (1933), prepared 5-methyltetrazole by this procedure.

(10) J. S. Mihina and R. M. Herbst, *J. Org. Chem.*, **15**, 1082 (1950), reported the conversion of nitriles to tetrazoles by heating them with hydrogen azide in a sealed tube.

(11) E. K. Harvill, R. M. Herbst and E. G. Schreiner, *ibid.*, **17** 1597 (1952).

of β-benzamidopropionitrile and the solution was heated under reflux for four days. The solvent was evaporated and the residue was extracted with 50 ml. of 1 *N* sodium hydroxide. The extract was made acidic with dilute hydrochloric acid and was concentrated to dryness under reduced pressure. The resulting solid was extracted with absolute ethanol which, in turn, was evaporated, and the residue was recrystallized from 10 ml. of water. There was obtained 0.2 g. of 5-β-benzamidoethyltetrazole. Approximately 9 g. of starting material was recovered.

5-β-Aminoethyltetrazole (I) Hydrochloride.—5-β-Benzamidoethyltetrazole (1.5 g.) suspended in 25 ml. of dilute hydrochloric acid was heated under reflux for 6 hours. Benzoic acid, which precipitated on cooling, was removed by filtration. The filtrate was evaporated to dryness under reduced pressure. After recrystallization from ethanol–ether, 5-β-aminoethyltetrazole hydrochloride was obtained in quantitative yield as prisms; m.p. 128–129°; pK'_a 5.0, 10.0 (66% dimethylformamide); no $\lambda_{max} > 210$ mμ; mol. wt., 152 (by titration).

Anal. Calcd. for $C_3H_7N_5 \cdot HCl$: C, 24.09; H, 5.39; Cl, 23.70. Found: C, 24.34; H, 5.95; Cl, 23.88.

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Biosynthesis and Characterization of 11β-Hydroxytestosterone[1]

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The introduction of an hydroxyl group at the 11-position of some steroid compounds by adrenal gland preparations has been reported. Hechter, *et al.*,[3] demonstrated this bioöxidation in the perfused adrenal gland. Other investigators[4,5] have obtained essentially the same results using brei, homogenates and more purified preparations.

Various steroids of the C_{21} and C_{19} types have been shown to undergo this bioöxidation. Consequently, it was considered of interest to investigate the potentiality of the adrenal gland to introduce a C_{11}-hydroxyl group in steroids which are recognized as major secretory products of other endocrine glands, since normally these compounds are present in the circulation and constitute potential substrates for this adrenocortical enzymatic system.

As a representative of these naturally occurring steroids, testosterone was perfused in homologous blood through isolated beef adrenal glands freshly obtained from the abattoir. In other experiments testosterone was incubated with fresh beef adrenal gland brei and homogenates. For the latter experiments the tissue was suspended in a modified Krebs–Ringer phosphate buffer at *p*H 7.4 (calcium ions were omitted), containing sodium fumarate and ATP as cofactors[6] and incubation was continued for two hours at 37° under an atmosphere of 95% oxygen and 5% carbon dioxide. The steroids were extracted from the incubation medium by dialysis according to a technique developed in these laboratories[7] and separated by paper chromatography.

Among several steroids isolated from the perfused medium and incubation mixture, one has been characterized as 11β-hydroxytestosterone (I), hitherto unreported in the literature.

The compound was purified by paper chromatography and crystallized twice from methanol–ether–pentane. White crystals were obtained which melted at 234.5–235.5°; $[\alpha]^{25}D$ 142° (2 mg. in 1.00 ml. of methanol). The infrared absorption spectrum of I is shown in Fig. 1.

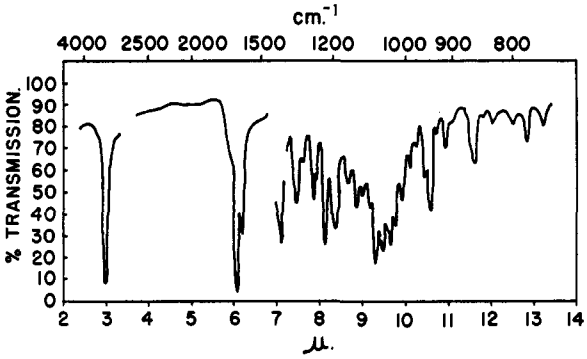

Fig. 1.—Infrared absorption spectrum of 11β-hydroxytestosterone: cell, 0.025 mm.; temp., 23°; concn., 3 mg. in 2 drops of Nujol.

Some characteristics of the compound are presented in Table I.

TABLE I

SOME CHARACTERISTICS OF 11β-HYDROXYTESTOSTERONE

Test	Result	Responsible structure
Modified Zimmerman	Blue	C_3-keto group[8,9]
Modified Lund	Orange	Δ^4-3-keto group[9]
Ultraviolet absorption maxima, mμ	242	α,β-Unsaturated ketone
Triphenyltetrazolium chloride	No reaction	Absence of reducin side chain
Rf value, benzene–formamide	0.07	.....

Chromic acid oxidation of I yielded adrenosterone (II) which was characterized by comparison with an authentic sample, showing the same chromatographic behavior in two different solvent systems, and identical color spot tests, sulfuric acid chromogen absorption spectrum, and ultraviolet

TABLE II

CHARACTERISTICS OF THE OXIDATION PRODUCTS OF 11β-HYDROXYTESTOSTERONE

Compound	Modified Zimmerman reaction	Modified Lund reaction	Ultraviolet absorption max., mμ	*Rf* value, benzene–formamide
II (adrenosterone)	Purple[a]	Orange	238	0.56
III	Blue	Orange	238	.24

[a] Indicative of a C_{17}-keto group.[8,9]

(1) This investigation was supported principally by a research grant from the Jane Coffin Memorial Fund for Medical Research and is based in part on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

(2) John Simon Guggenheim Memorial Foundation Fellow.

(3) O. Hechter, R. Jacobsen, R. Jeanloz, H. Levy, C. N. Marshall, G. Pincus and V. Schenker, THIS JOURNAL, **71**, 3261 (1949), and *Arch. Biochem.*, **25**, 457 (1950).

(4) K. Savard, A. A. Green and L. A. Lewis, *Endocrinology*, **47**, 418 (1950).

(5) M. Hayano and R. I. Dorfman, *J. Biol. Chem.*, **201**, 175 (1953).

(6) M. Hayano, R. I. Dorfman and R. V. Yamada, *ibid.*, **192**, 175 (1951).

(7) L. R. Axelrod and A. Zaffaroni, unpublished data.

(8) C. D. Kochakian and G. Stidworthy, *J. Biol. Chem.*, **199**, 607 (1952).

(9) L. R. Axelrod, *ibid.*, in press.