

Revised Structure of the Adrenochrome Sulfonate, Ac-17 and Ac-44

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Ac-17 is a water soluble adrenochrome sulfonate, which was synthesized from adrenochrome semicarbazone by treatment with aqueous sodium bisulfite. Ac-44 is an isomer of Ac-17, and which was obtained when epinine sulfonic acid (2) was oxidized by potassium ferricyanide in an alkaline solution and then reacted with semicarbazide. Ac-17 has been widely employed as an effective hemostatic for clinical use since 1957.

Previously the structure of Ac-17 had been assigned as sodium 1-methyl-5-semicarbazono-6-oxo-2,3,5,6-tetrahydroindole-3-sulfonate by Iwao (3) and that of Ac-44 had been demonstrated as sodium 1-methyl-5-semicarbazono-6-oxo-2,3,5,6-tetrahydroindole-2-sulfonate by Tomino (4). Now we wish to report the revised structure of both compounds.

The epinine sulfonate (2) was prepared from epinephrine by refluxing with aqueous sodium bisulfite. This sulfonate was independently synthesized and the structure

was revealed as sodium 2-methylamino-1-(3,4-dihydroxyphenyl)ethylsulfonate by Higuchi *et al.* (5), (6), (7), whereas Tomino assigned the structure as sodium 1-methylamino-2-(3,4-dihydroxyphenyl)ethylsulfonate (4).

In order to elucidate this discrepancy, *l*-epinephrine was refluxed with aqueous sodium bisulfite at pH 6.5 for 3 hours. An optically active epinine sulfonate ($[\alpha]_D^{23} +27^\circ$, $C = 0.2$, water) was obtained in good yield. This result indicated that the reasonable structure for the epinine sulfonate was that of epinine-1-sulfonate. Although there remained little possibility of another structure, spectrophotometrical analysis using the deuterated compounds was carried out to confirm the structure.

Adrenalone hydrochloride I in deuterium oxide was reduced with deuterium to give deuterio-epinephrine II^D. Sodium deuterio-epinine sulfonate III^D was obtained in 70% yield when II^D was heated with sodium bisulfite in deuterium oxide. The mass spectrum of II^D is given in Table I.

The nmr spectra of the deuterated compounds indicate that the two aromatic hydrogens are exchanged by deuterium. The multiplet at τ 5.6 (III) is assigned to the benzyl hydrogen, and the multiplet at τ 6.3 (2H) is attributed to the β -methylene hydrogens of III. In the nmr spectrum of III^D, the signal of the benzyl hydrogen disappears and the quartet, of which the coupling constant ($J = 13$ Hz) is a characteristic of geminal protons, appears at τ 6.24, as expected. Since the uv spectrum of III (λ max 228, 280 m μ) indicates that the sulfonate group is on the side chain, the structure of III is thus determined as sodium 2-methylamino-1-(3,4-dihydroxyphenyl) ethyl sulfonate.



The structure of Ac-44 obtained by the oxidative cyclization of III is therefore assigned as sodium 1-methyl-5-semicarbazono-6-oxo-2,3,5,6-tetrahydroindole-3-sulfonate unless some otherwise unexpected rearrangement of the sulfonate group may occur during the oxidation.

Deuterium substituted Ac-17 VI^D was synthesized from II^D via deuteroadrenochrome semicarbazone VD

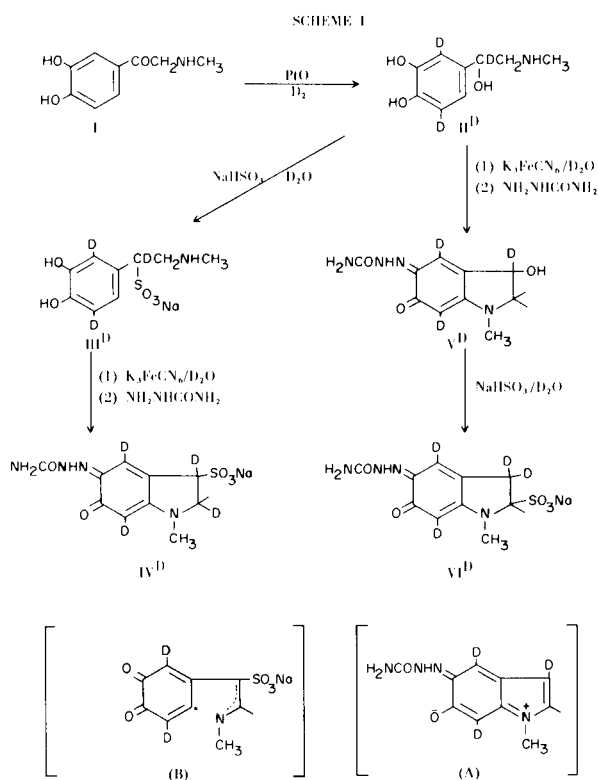


TABLE I

Mass Spectra of the Deuterated Compounds and the Corresponding Non-deuterated Compounds

NO		m/e at 45 ev								
II	M +	183(9)(a)	165(13)	139(11)	124(20)	93(23)	65(21)	44(100)		
II ^D	M +	186(9)	168(9)	142(14)	127(13)	96(22)	68(26)	44(100)		
V	M +	236(13)	218(10)	192(83)	174(35)	164(88)	147(33)	119(33)	91(67)	43(100)
V ^D	M +	239(2)	221(2)	195(13)	177(18)	167(21)	150(31)	121(17)	91(43)	43(100)

(a) () = relative intensity.

TABLE II

The Nmr Spectra of the Deuterated Compounds and the Corresponding Non-deuterated Compounds (τ)

Compound NO	Aromatic H	C ₃ -H (a)	C ₂ -H (a)	N-CH ₃
II	3.10	5.08	6.80	7.24
(CF ₃ COOH-D ₂ O)	(m, 3H)	(t, 1H)	(d, 2H)	(s, 3H)
II ^D	3.15	-----	6.80	7.25
(CF ₃ COOH-D ₂ O)	(s, 1H)		(s, 2H)	(s, 3H)
III	3.03	Ca _{5,6}	Ca _{6,3}	7.23
(D ₂ O)	(m, 3H)	(1H, ABX)	(2H, ABX)	(s, 3H)
III ^D	3.02	-----	6.24	7.22
(D ₂ O)	(s, 1H)		(q, 2H)	(s, 3H)
VI	3.06, 4.16	6.45	4.76	6.49
(D ₂ O, 70°)	(s, 1H)(s, 1H)	(d, 2H)	(t, 1H)	(s, 3H)
VI ^D	-----	-----	4.80	6.48
(D ₂ O, 70°)			(s, 1H)	(s, 3H)
IV	3.22, 4.62	Ca _{5,55}	Ca _{5,9}	6.93
(D ₂ O)	(s, 1H)(s, 1H)	(m, 1H)	(m, 2H)	(s, 3H)
IV ^D	-----	-----	5.88	6.92
(D ₂ O)			(s, 1H)	(s, 3H)

(a) Expedient numbering for II and III.

under reaction conditions similar to those of the non-deuterated compounds. The mass spectrum of V^D is compared with that of V, which is also given in Table I. The nmr of VI shows a triplet at τ 4.76 (1H) and a doublet at τ 6.45 (2H). On the other hand, only one singlet at τ 4.80 (1H) appears in the nmr spectrum of VI^D except for a singlet at τ 6.48 (3H) which is assigned to the *N*-methyl hydrogens.

This result indicates that the sulfonate group of VI is on C₂ of the indole ring, because if the sulfonate group were bound at C₃, a signal for methylene hydrogens of C₂ must appear in the region τ 5.0 ~ 7.0. The singlet at τ 4.80 also supports this determination. It is suggested that VI^D may be formed *via* an intermediate (A) shown in Fig. 1 since the hydrogen on C₃ has been exchanged for deuterium during the reaction.

Deuterium-substituted Ac-44 IV^D was also synthesized from III^D for further confirmation of the structure.

Unfortunately, a fine nmr spectrum in deuterium oxide could not be obtained because of limited solubility, compound IV was therefore converted to its ammonium salt for this purpose.

A multiplet at τ 5.5 for the proton of C₃ in IV, is absent in the nmr spectrum of IV^D. The singlet at τ 5.8 (1H) suggests the reaction intermediate (B) shown in Fig. 1.

As a result of these spectrophotometrical studies, it is concluded that the structure of Ac-17 should be revised as sodium 1-methyl-5-semicarbazono-6-oxo-2,3,5,6-tetrahydroindole-2-sulfonate and that of Ac-44 as sodium-1-methyl-5-semicarbazono-6-oxo-2,3,5,6-tetrahydroindole-3-sulfonate.

EXPERIMENTAL

Melting points are uncorrected. The ir spectra were recorded

on a Hitachi 215 and the nmr spectra were recorded on a JEOL MH-60 spectrometer using tetramethylsilane as an internal standard. Optical rotations were measured with JASCO-DIP-180 polarimeter.

Deuterium-Substituted Epinephrine (II^D).

A solution of adrenalone hydrochloride I (2 g.) in 10 ml. of deuterium oxide was reduced with deuterium in the presence of 100 mg. of platinum oxide at room temperature. The catalyst was filtered off, the mother liquid was made alkaline with ammonia. The crystals which separated were collected and washed with cold water, m.p. 192-193° dec.; yield 1.2 g.; ν max (Nujol): 3250 (OH).

Deuterium-Substituted Adrenochrome Semicarbazone (V^D).

Into a solution of 500 mg. of II^D in 8 ml. of deuterium oxide containing 0.3 ml. of acetic acid, a solution of deuterium oxide (ca. 12 ml.), containing 3.62 g. of potassium ferricyanide and 0.25 g. of sodium bicarbonate, was added dropwise within 50 seconds at 10 ~ 15°.

After 1 minute, 0.9 g. of semicarbazide hydrochloride and 0.68 g. of sodium bicarbonate were added to the reaction mixture. The stirring was continued for 30 minutes at room temperature. The crystals which separated were collected, washed with a small amount of deuterium oxide followed by acetone and dried. Orange crystals of V^D (200 mg.) were obtained, m.p. 200°; ν max (Nujol): 3320 (OH), 2570, 2520, 1673 (CO) 1640 (NHCO) cm^{-1} .

Deuterium-Substituted Ac-17 (VI^D).

A mixture of 200 mg. of V^D and 260 mg. of sodium bisulfite in 2 ml. of deuterium oxide was stirred for 5 hours at 53 ~ 57°.

The undissolved material was filtered off and the mother liquid was allowed to stand for 8 hours in a refrigerator. The yellow needles which separated were collected and washed with acetone. Thirty two mg. of VI^D was obtained, m.p. 219 ~ 220° dec., ν max (Nujol): 3400 (NH), 1680 (CO) 1640 (NHCO) 1190, 1050 (SO₃-).

Deuterium-Substituted Epinephrine Sulfonate (III^D).

A mixture of 300 mg. of II^D and 180 mg. of sodium bisulfite in 4 ml. of deuterium oxide was heated for 3 hours at 80 ~ 90° (pH 7.0). The pH was then adjusted at 6.5 with sulfur dioxide to give a clear solution.

The reaction mixture was condensed to 0.3 ml. *in vacuo* and allowed to stand overnight in a refrigerator. The crystals which separated were collected, washed with cold deuterium oxide, acetone and ether. Two-hundred and fifty mg. of III^D was obtained, m.p. 245 ~ 247° dec.; ν max (Nujol): 3400 ~ 3100 (OH) 1160, 1050 (SO₃-).

Deuterium-Substituted Ac-44 (IV^D).

Into a solution of 180 mg. of III^D in deuterium oxide, a solution of 750 mg. of potassium ferricyanide and 57 mg. of sodium bicarbonate in 2.1 ml. of deuterium oxide was added dropwise at 15°.

After 5 minutes, a solution of 150 mg. of semicarbazide hydrochloride and 110 mg. of sodium bicarbonate in 0.26 ml. of deuterium oxide was added and the mixture was allowed to stand for 30 minutes at 20°. The crystals of IV^D were obtained as yellow needles, yield 60 mg., m.p. 224-226° dec.; ν max (Nujol): 3400 (NH) 1670 (CO) 1640 (-NHCO) 1205, 1050 (SO₃-).

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