252. *The Structure of Adreno-cortical Metabolites: $\Delta^{9:11}$-Androstene-3 : 17-dione.*

By C. W. Shoppee.

11(β)-Hydroxy*iso*androsterone (II), obtained from the adrenal cortex of cattle, and 11(β)-hydroxyandrosterone (VIII), isolated by Mason and Kepler (*J. Biol. Chem.*, 1945, **161**, 235) from the urine of human subjects with adrenal dysfunction, by dehydration afford epimeric $\Delta^{9:11}$-androsten-3-ol-17-ones (III) (IX), which are oxidised by chromium trioxide to $\Delta^{9:11}$-androstene-3 : 17-dione (IV). The Δ^{11}-androsten-3(α)-ol-17-one (VII), isolated by Fieser *et al.* (*J. Amer. Chem. Soc.*, 1941, **63**, 582) from the urine of a girl suffering from an adreno-cortical tumour furnishes by oxidation a diketone, isomeric and not identical with (IV), which is regarded as Δ^{11}-androstene-3 : 17-dione (X). The origin of 17-ketosteroids in general and of (VII) and (VIII) in particular is briefly considered.

Some years ago the writer described (*Helv. Chim. Acta*, 1940, **23**, 740) a degradation of the pentol (I; Reichstein's substance A) isolated from the adrenal cortex of cattle; oxidation of (I) with periodic acid gave androstane-3(β) : 11(β)-diol-17-one (II), a compound also isolated from the cortex (Reichstein and von Euw, *ibid.*, 1938, **21**, 1197, 1201), and dehydration of the 3-monoacetate of this with hydrochloric acid eliminated the 11(β)-hydroxyl group to afford $\Delta^{9:11}$-androsten-3(β)-ol-17-one acetate (III; R = Ac), which by hydrogenation and acetylation furnished androstane-3(β) : 17(α)-diol diacetate.

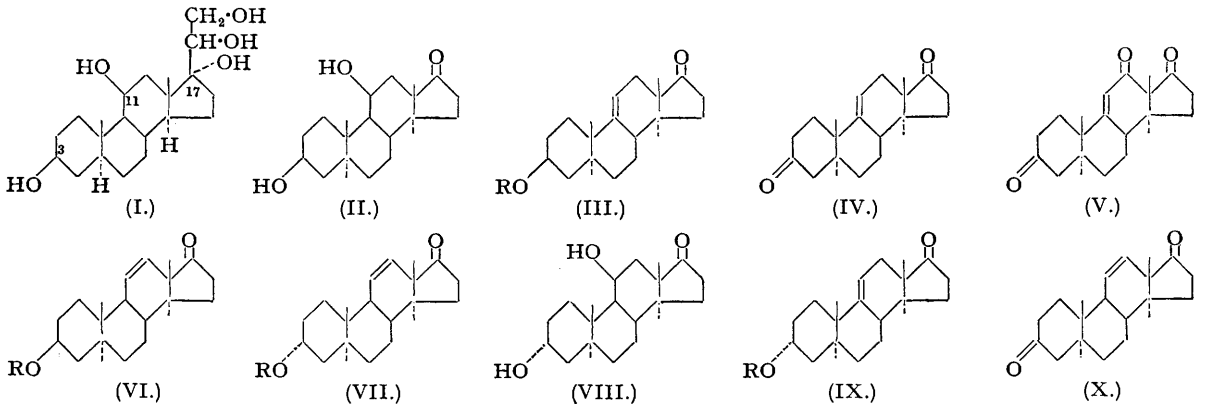
The acetate (III; R = Ac) was originally assigned the Δ^{11}-structure (VI; R = Ac), but subsequent work * indicates by analogy that the $\Delta^{9:11}$-formulation now employed is correct.

The acetate (III; R = Ac) has been hydrolysed to the free *hydroxy-ketone* (III; R = H) and this converted by oxidation with chromium trioxide to $\Delta^{9:11}$-*androstene*-3 : 17-*dione* (IV; m. p. 154°, $[\alpha]_D^{14°}$ +156° ±2·5°, $[\alpha]_{5461}^{14°}$ +183° ±2·5°). The diketone (IV) was accompanied by traces of a higher-melting substance which may be $\Delta^{9:11}$-androstene-3 : 12 : 17-trione (V); if the identity of this by-product could be established as (V), this would provide further evidence in support of the position assigned to the double bond in (III) and (IV).

In 1941, Wolfe, Fieser, and Friedgood (*J. Amer. Chem. Soc.*, 1941, **63**, 582) isolated from the urine of a girl suffering from a recurrent adreno-cortical tumour an androstenolone which they suggested might be the C_3-epimeride (VII; R = H) of the supposed hydroxy-ketone (VI; R = H), since by hydrogenation it furnished

* The compound obtained by dehydration of 11(β)-hydroxyprogesterone with hydrochloric acid and originally described as Δ^{11}-progesterone (Shoppee, *Helv. Chim. Acta*, 1941, **24**, 351) is to be regarded as $\Delta^{9:11}$-progesterone because it is different from the true Δ^{11}-progesterone obtained by pyrolysis of 12(β)-benzoxyprogesterone (Reichstein and Hegner, *ibid.*, 1943, **26**, 715). Further, dehydration of methyl 11(β)-hydroxycholanate (Reichstein and Reich, *ibid.*, 1943, **26**, 568) and of methyl 3(α) : 11(β)-dihydroxycholanate (Reichstein and Lardon, *ibid.*, p. 586) with hydrochloric acid produces only traces of the Δ^{11}-esters, the main products being the $\Delta^{9:11}$-esters whose structures were proved by conversion with perbenzoic acid into the known 9 : 11-oxidoesters; compare also Reichstein and Lardon (*ibid.*, 1945, **28**, 1420).

androstane-3(α) : 17(α)-diol; they also suggested the possibility that the preliminary hydrolysis with hydrochloric acid, to which the urine had been subjected, had caused dehydration of a cortical metabolite (VIII).



The level of urinary 17-ketosteroids is largely unaltered by castration, but rises when the adrenal gland is affected by benign hyperplasia or by malignant tumour of the cortex; further, in Addison's disease, which results from destruction of the adrenal gland, generally following tubercular infection, abnormally low values are found. It therefore seems probable that urinary 17-ketosteroids arise principally from the adrenal cortex rather than the gonads, and represent end-products of the metabolism of the adrenal cortical hormones and their congeners.

Accordingly, it appeared of interest to attempt to establish the structure of the androstenolone of Wolfe, Fieser, and Friedgood, and this was suggested to Professor Fieser in 1941. Various circumstances combined to preclude dealing with the matter until lately; in the meantime Mason and Kepler (*J. Biol. Chem.*, 1945, **161**, 235) have isolated androstane-3(α) : 11(β)-diol-17-one (VIII) from the urine of 3 out of 6 women suffering from tumour of the adrenal cortex and from the urine of 3 out of 4 patients suffering from bilateral andrenal cortical hyperplasia,* and have established its constitution by oxidation with chromium trioxide to androstane-3 : 11 : 17-trione. Thus the 11(β)-hydroxyl group survived the preliminary hydrolysis with N-hydrochloric acid, although Wolfe, Fieser, and Friedgood, using only approximately 0·1N-acid, had been unable to detect (VIII). Nevertheless it was anticipated that (VIII) by loss of the 11(β)-hydroxyl group might yield Wolfe, Fieser, and Friedgood's compound (VII; R = H, m. p. 181—183°, $[\alpha]_D^{26.5°}$ +122° ±2°; R = Ac, m. p. 178—180°, $[\alpha]_D^{26.5°}$ +114° ±5°), but application by Mason and Kepler of the writer's method for the elimination of the 11(β)-hydroxyl group characteristic of the cortical substances furnished an isomeric androstenolone (m. p. 189—190°, $[\alpha]_D^{26°}$ +140 ±2°; acetate, m. p. 190—192°, $[\alpha]_D^{25°}$ +135° ±3°). Since this new isomeride by hydrogenation afforded androstane-3(α) : 17(α)-diol, the isomerism is conditioned solely by the location of the double bond. Through the kindness of Dr. H. M. Mason, who provided 15 mg. of (VIII), it has now been shown that the androstenolone of Mason and Kepler is the C_3-epimeride (IX; R = H) of the cortical degradation product (III; R = H) because by oxidation with chromium trioxide it yields the same unsaturated diketone (IV).

Quite recently, Miller, Dorfman, and Sevringhaus (*Endocrinology*, 1946, **38**, 19) have isolated the cortical metabolite (VIII) from a pathological urine which had not been subjected to preliminary acid hydrolysis. Dorfman, Schiller, and Sevringhaus (*ibid.*, 1945, **37**, 262) had previously obtained from hydrolysed urine of the same patient an androsten-3(α)-ol-17-one acetate, m. p. 179—182°, which was considered possibly to be identical with the compound (VII; R = Ac), m. p. 178—180°, of Wolfe, Fieser, and Friedgood; the m. p. of this acetate has now been raised to 188·5—189°, and it has been shown to be identical with the acetate (IX; R = Ac), m. p. 190—192°.

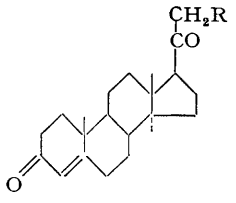
With the kind co-operation of Professor Fieser, who provided material furnishing 6 mg. of (VII; R = H), a corresponding oxidation has been carried out on this compound. The 3(α)-benzoyl group of the benzoate (VII; R = Bz) showed unexpected resistance to hydrolysis by potassium carbonate, and in part survived treatment with cold N/5-sodium hydroxide; oxidation of (VII; R = H) with chromium trioxide gave an *androstene*-3 : 17-*dione*, m. p. 145—146°, which is provisionally regarded as (X), although location of the double bond at other positions, *i.e.*, Δ^6 or Δ^7, is not excluded by the chemical evidence adduced by Wolfe, Fieser, and Friedgood in respect of its precursor (VII; R=H). The new diketone gives little or no m. p. depression on admixture with $\Delta^{9:11}$-androstene-3 : 17-dione (IV), but its specific rotation, $[\alpha]_D^{15°}$ + 141° ± 3·5°, $[\alpha]_{5461}^{15°}$ + 170·5° ± 3·5°, clearly indicates that it is different from this substance.

Since progesterone (XI; R = H) (Venning and Brown, *Endocrinology*, 1937, **21**, 711) and deoxycorticosterone (XI; R = OH) (Westphal, *Z. physiol. Chem.*, 1942, **273**, 13; Hoffman, Kazmin, and Browne, *J. Biol. Chem.*, 1943, **147**, 259) undergo metabolic reduction to pregnane-3(α) : 20(α)-diol (XII) (rings A/B, *cis*), the

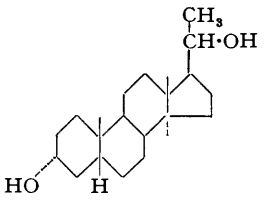
* Note (added June 14th, 1946) : Mason (*J. Biol. Chem.*, 1946, **162**, 745) reports the isolation of (VIII) from the urine of normal men, but in about one fifth the quantity obtained from pathological urines.

metabolic production of the androstane derivative (VII; R = H) (cf. also VIII) (rings A/B, *trans*) may indicate that it originates from an 11(β)-hydroxy-cortical hormone, *e.g.*, corticosterone (XIII) or 17(α)-hydroxycorticosterone (XIV), because it is known (Kendall, Mason, Hoehn, and McKenzie, *J. Biol. Chem.*, 1937, **120**, 719; Steiger and Reichstein, *Helv. Chim. Acta*, 1937, **20**, 817; 1938, **21**, 161, 828; cf. Shoppee, *ibid.*, 1941, **24**, 351) that reduction *in vitro* of 11(β)-hydroxy-Δ^4-3-keto-steroids furnishes exclusively compounds of the androstane series and because the orientating influence of an 11(β)-hydroxyl-group on the stereochemical course of reduction might be expected also to operate *in vivo*.

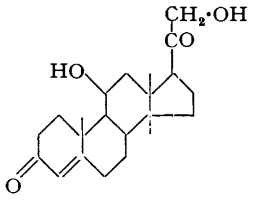
It may be remarked that the cortical metabolite (VIII) and Fieser's compound (VII; R = H), both derived from pathological urines, are 3(α)-hydroxy compounds; of the 27 steroids so far isolated from the adrenal cortex of presumably normal cattle, all the 13 saturated members are 3-hydroxy compounds of the *allo*-series, but, of these 13 individuals, 12 possess the (β)-configuration in respect of the hydroxyl group at C_3 and one only (Reichstein's substance C) the (α)-configuration.



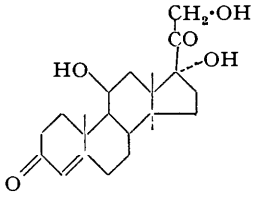
(XI.)



(XII.)



(XIII.)



(XIV.)

Experimental.

(All m. ps. were determined thermoelectrically on a Kofler block and are corrected: limit of error ±2°. All solvents used for chromatographic analysis were rigorously purified and dried.)

$\Delta^{9:11}$-*Androsten*-3(β)-*ol*-17-*one* (III; R = H).—The acetate (III; R = Ac) (27·5 mg.) was dissolved in methanol (2 c.c.), a solution of potassium carbonate (23 mg.) in water (0·5 c.c.) added, and the mixture kept for 48 hours at 20°. The long thin plates which separated were filtered off, washed with a little water, and dried (12 mg.; m. p. 169—172°). The material sublimed readily in a molecular flask at 130° (bath temp.)/0·01 mm., and was recrystallised from dilute methanol to give 9 mg. of $\Delta^{9:11}$-*androsten*-3(β)-*ol*-17-*one*, m. p. 170—172·5°, $[\alpha]_D^{13°}$ +125·5° ±2°, $[\alpha]_{5461}^{13°}$ +161° ±2° (*c*, 0·534 in ethanol) [Found (after drying at 40°/0·01 mm.): C, 79·40; H, 9·88. $C_{19}H_{28}O_2$ requires C, 79·13; H, 9·78%]. The aqueous methanol filtrate and mother liquor were united (methanol removed under reduced pressure) and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and concentrated to afford 13 mg. of the hydroxy-ketone, m. p. 169—171°.

$\Delta^{9:11}$-*Androstene*-3:17-*dione* (IV).—(a) *From* (III: R = H). The hydroxy-ketone (III; R = H) (18 mg., m. p. 169—172°) was dissolved in pure acetic acid (0·35 c.c., redistilled over chromium trioxide), and a 2% solution of chromium trioxide in acetic acid (0·35 c.c. ≡ 7 mg. CrO_3) added. The solution became turbid, but was clear after 16 hours at 15°. After complete removal of acetic acid under reduced pressure at 30°, a few drops of water were added and the product was extracted with ether; the aqueous solution contained an excess of chromium trioxide. The ethereal extract was washed with N-sulphuric acid, water, N-sodium carbonate, and again with water, dried briefly (Na_2SO_4), and evaporated. The crystalline residue separated from the ether–pentane in thin prisms, which were washed with ether–pentane (1 : 1) and pentane: 15 mg., m. p. 150—155° to a turbid melt clearing at about 160°. The mother liquor by evaporation gave 2 mg. of non-crystalline material.

The crystalline product (15 mg.) was purified by dissolution in benzene–pentane (1 : 2; 2 c.c.) and filtration through a column of aluminium oxide (Merck–Brockmann, Activity III *; 0·5 g.) prepared in pentane (5 c.c.). Each eluate (3 c.c.) was evaporated, the residue was crystallised from ether–pentane, and the crystals were washed with ether–pentane and pentane.

Fraction.	Eluant.		Eluate.
1—3	Pentane		—
4—9	Benzene–pentane	(1 : 1)	Prisms, m. p. 152—154°.
10	Benzene		,, ,, 153—154°.
11, 12	,,		Traces of cryst. material, m. p. 150—154°.
13	Ether–benzene	(1 : 9)	—
14	,,	(1 : 4)	Little cryst. material, m. p. 190—195° after softening from 180°.
15	,,	(1 : 1)	Trace of cryst. material.
16	Ether		—
17	Acetone–ether	(1 : 1)	—

Fractions 4—12 were united and recrystallised from ether–pentane to furnish $\Delta^{9:11}$-*androstene*-3:17-*dione* (10 mg.); two crystal habits were observed, large flat plates and long thin prisms, both melting at 153—154°, the melt recrystallising at 148° and remelting at 154°, $[\alpha]_D^{14°}$ +156° ±2·5°, $[\alpha]_{5461}^{14°}$ +183° ±2·5° (*c*, 0·404 in ethanol) [Found (after drying at 40° in a high vacuum): C, 79·34; H, 9·34. $C_{19}H_{26}O_2$ requires C, 79·67; H, 9·15%].

(b) *From* (VIII). Androstane-3(α):11(β)-diol-17-one (VIII) (14 mg., m. p. 198°), dehydrated by the procedure described by Mason and Kepler (*loc. cit.*), furnished $\Delta^{9:11}$-androsten-3(α)-ol-17-one (IX; R = H) (2 mg.), hexagonal prisms, m. p. 189—190° and its acetate (IX; R = Ac) (13 mg.), long thin prisms, m. p. 192—193°. The acetate (13 mg.) by hydrolysis gave the free hydroxy-ketone (11·5 mg.), m. p. (crude) 188—190°. The hydroxy-ketone (13·5 mg.) was dissolved in pure acetic acid (0·25 c.c., redistilled over chromium trioxide) and treated with a 2% solution of chromium trioxide in acetic acid (0·25 c.c. ≡ 5 mg. CrO_3); the solution remained clear and was kept for 16 hours at 20°. Working up showed an excess of chromium trioxide to be present, and afforded a crystalline residue (12·7 mg.), m. p. (crude) 145—151°. This residue was dissolved in benzene–pentane (1 : 1) (0·5 c.c.) and introduced on to a column of aluminium

* According to the scale suggested by Brockmann and Schodder (*Ber.*, 1941, **74**, 73).

oxide (Merck–Brockmann, activity III; 0·5 g.) prepared in pentane (5 c.c.). Elution with 7 × 2 c.c. portions of benzene–pentane (1 : 1) and 2 × 2 c.c. portions of benzene yielded $\Delta^{9:11}$-androstene-3 : 17-dione (8·3 mg.), thin prisms from ether–pentane, m. p. 153—154°, mixed m. p. with the specimen prepared by method (a), 153—154°, the melt resolidifying at 150° and remelting at 154°, $[\alpha]_D^{15°}$ +154·5° ±2°, $[\alpha]_{5461}^{15°}$ +181·3° ±2° (*c*, 0·634 in ethanol) [Found (after sublimation at 140°/0·01 mm.): C, 79·50; H, 9·20%]. Elution with ether–benzene (1 : 4) gave traces of a substance, crystallising from ether–pentane in plates, m. p. 195—197°, which may be $\Delta^{9:11}$-androstene-3 : 12 : 17-trione; the quantity was insufficient for analysis.

$\Delta^{11(?)}$-Androsten-3(a)-ol-17-one (VII; R = H).—(*a*) The acetate (VII; R = Ac) (2·6 mg.) was dissolved in methanol (0·5 c.c.), a solution of potassium carbonate (2·5 mg.) in water (0·05 c.c.) was added, and the mixture kept at 20° for 48 hours. After evaporation under reduced pressure, the residue was extracted with ether and furnished a mixture of the hydroxy-ketone and the unhydrolysed acetate.

(*b*) The benzoate (VII; R = Bz) (3·4 mg.; m. p. 164°) was similarly treated, but crystallised out in long prisms, m. p. 164—165°, which could not be redissolved by gentle warming. After addition of methanol (0·5 c.c.), the mixture was therefore refluxed on the steam-bath for 1 hour. Working up gave a residue which crystallised on being kept overnight, m. p. 140° approx. This product, consisting of the hydroxy-ketone and unhydrolysed benzoate, was united with the crude hydroxy-ketone obtained under (*a*) to give 6 mg. of material, which was introduced in benzene (0·25 c.c.) on to a column of aluminium oxide (Merck–Brockmann, activity III; 0·5 g.) prepared in pentane (5 c.c.). Elution with benzene–pentane (1 : 1) (5 × 2 c.c.) and with benzene (3 × 2 c.c.) removed all unhydrolysed acetate and benzoate. Crystallisation of these united fractions from ether–pentane furnished prisms, m. p. 160—162°, m. p. 162—164° on admixture with the benzoate; the crystals and the mother liquor gave 4 mg. of unhydrolysed material, which was again submitted to hydrolysis (*vide infra*). Elution of the column with ether–benzene (1 : 1) (2 × 2 c.c.) and with ether (3 × 2 c.c.) yielded fractions which crystallised spontaneously by evaporation; they were united and the residue recrystallised from ether–pentane to afford 1·4 mg. of long needles, m. p. 178—180°, m. p. 178—181° on admixture with a genuine specimen of the hydroxy-ketone (VI; R = H).

The unhydrolysed material (4 mg.) was dissolved in methanol (1·5 c.c.), N-sodium hydroxide (0·3 c.c.) added, and the mixture kept for 16 hours at 20°. After addition of a few drops of water, the solution was saturated with carbon dioxide, methanol removed under reduced pressure, and the suspension extracted with ether. The extract was washed with a little water, dried briefly (Na_2SO_4), and evaporated to yield an oil (3 mg.) which crystallised partially. This material was chromatographed as described above on aluminium oxide (0·3 g.). Elution with benzene–pentane (1 : 1) gave only mere traces of crystalline material; the first benzene eluate gave a small crystalline residue, which consisted of unhydrolysed benzoate, m. p. 164—165° after recrystallisation from ether–pentane. m. p. 135—140° on admixture with the hydroxy-ketone. Subsequent benzene eluates yielded no residue. Elution with ether–benzene (1 : 1) furnished a residue which crystallised spontaneously, m. p. 170—172°, whilst the first ether eluate similarly afforded crystals, m. p. 172—173°; these fractions were united and recrystallised from ether–pentane to give the hydroxy-ketone (VII; R = H) (1·6 mg.), m. p. 174°, resolidifying immediately and remelting at 174°, and giving no depression on admixture with a genuine specimen, m. p. 178°. Subsequent ether eluates yielded no residue.

$\Delta^{11(?)}$-Androstene-3 : 17-dione (X).—The hydroxy-ketone (VII; R = H) obtained by the foregoing hydrolyses (3 mg.) together with the genuine specimen supplied by Professor Fieser (m. p. 178—181°; 3·5 mg.) was dissolved in acetic acid (0·12 c.c., redistilled over chromium trioxide), and a 2% solution of chromium trioxide in acetic acid (0·12 c.c. ≡ 2·4 mg. CrO_3) added. The turbid solution became clear in about ½ hour, and was kept for 16 hours at 15°. After complete removal of acetic acid under reduced pressure at 30°, a few drops of water were added, and the product was extracted with ether; the aqueous solution contained an excess of chromium trioxide. The ethereal extract was washed with N-sulphuric acid, water, N-sodium carbonate, and again with water, dried briefly (Na_2SO_4), and evaporated to furnish a crystalline residue (6 mg.), m. p. 139—141° after some softening. This product was dissolved in benzene (0·25 c.c.) and analysed chromatographically on a column of aluminium oxide (Merck–Brockmann, activity III; 0·25 g.) prepared in pentane (5 c.c.) as follows:

Fraction.	Eluant.	Volume, c.c.	Eluate.
1	Pentane	5	Trace of oil.
2	,,	5	—
3	Benzene–pentane (1 : 9)	1	—
4	,, (1 : 4)	1	—
5	,, (1 : 2)	1	Traces of cryst. material.
6	,, (1 : 1)	2	Cryst. spontaneously, m. p. 144—146°.
7	,, (1 : 1)	2	,, ,, ,, 140—142°.
8	,, (1 : 1)	2	,, ,, ,, 140—142°.
9	Benzene	2	,, ,, ,, 139—141°.
10	,,	2	—
11	Ether	5	—

Fractions 5—9 were united to give 5 mg. of material, which by recrystallisation from ether–pentane furnished *$\Delta^{11(?)}$-androstene-3 : 17-dione* (3 mg.) as clusters of prisms, m. p. 145—146°, recrystallising at once on slight cooling and remelting at 146°. On admixture with $\Delta^{9:11}$-androstene-3 : 17-dione, m. ps. 143—146° and 143—147° with immediate crystallisation of the melt on slight cooling and remelting at 146—147° were observed, so that these substances give little or no depression. The specific rotation, however, confirms their non-identity: $[\alpha]_D^{15°}$ +141·5° ±3·5°, $[\alpha]_{5461}^{15°}$ +170·5° ±3·5° (*c*, 0·2932 in ethanol). For analysis, the material used for the determination of the specific rotation was recovered and sublimed at 130°/0·001 mm. (Found: C, 79·20; H, 9·24. $C_{19}H_{26}O_2$ requires C, 79·67; H, 9·15%).

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