445. *Studies in the Sterol Group. Part LIII.* The Preparation of 7-Hydroxy-3-keto-Δ^4-steroids.*

By C. W. Greenhalgh, H. B. Henbest, and E. R. H. Jones.

7β-Hydroxycholest-4-en-3-one can be prepared in rather low yield by Oppenauer oxidation of 7β-hydroxycholesterol; attempts to prepare the 7α-epimer were not successful owing to its ready dehydration. Lithium aluminium hydride reduction of the enol methyl ether of "3 : 7-diketocholestene" has been shown to afford cholesta-4 : 6-dien-3-one in 60% yield—the course of this reaction indicates the methyl ether to have the structure, 3-methoxycholesta-3 : 5-dien-7-one.

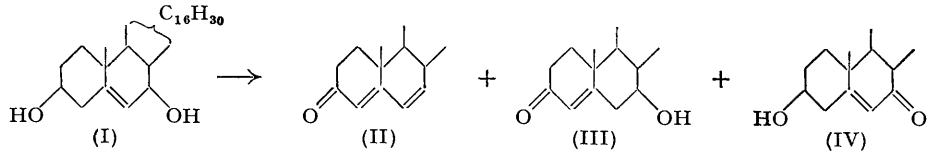
The lack of antirheumatic activity shown by Reichstein's compound S, and related compounds not containing an 11-oxygen substituent, indicates that an oxygen group in this position is a prerequisite for certain types of biological activity. However, 11-deoxy-compounds related to cortisone, which carry instead an oxygen substituent at a nearby position in the "centre" of the steroid molecule, have not been prepared or tested. Thus it might prove possible to replace the $C_{(11)}$-substituent by one at one of the positions 7, 8, and 9, and still retain some degree of antirheumatic activity. Position 7 is of particular interest because a variety of substituents can be introduced into this position starting from Δ^5-steroids, although only one such compound containing the physiologically important 3-keto-Δ^4-system, 7α-methoxycholest-4-en-3-one,† has yet been described (Henbest and Jones, *J.*, 1948, 1798). Some further 7-methoxy-compounds are described in the following paper, but since a 7-hydroxyl group might offer advantages over a methoxy-group with regard to biological activity, the preparation of this type of compound (III) has now been studied in the cholesterol series.

Oppenauer oxidation of 7β-hydroxycholesterol (I) afforded a gum, the light absorption of which indicated the presence of an αβ-unsaturated ketone and an αβ : γδ-diene-ketone. Chromatography yielded three crystalline compounds, cholesta-4 : 6-dien-3-one (II) (40%), 7β-hydroxycholest-4-en-3-one (III) (10%), and 7-ketocholesterol (IV) (25%). The relative proportions of these compounds suggests a preference for oxidation to occur at the less sterically hindered 3-position than at the more activated 7-position. The constitution (III) for the new hydroxy-ketone was confirmed by its ultra-violet light absorption, and by the preparation of an acetate and a benzoate. An attempt to prepare this benzoate by Oppenauer oxidation of 7β-benzoyloxycholesterol gave cholesta-4 : 6-dien-3-one (II).

* Part LII, *J.*, 1951, 2402.

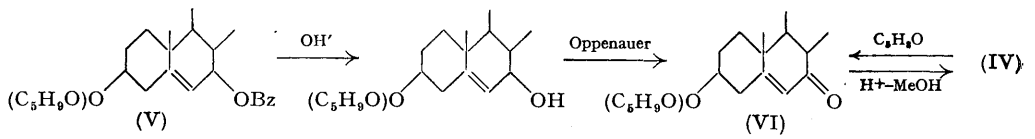
† Following the suggestions of Fieser, Fieser, and Chakravarti (*J. Amer. Chem. Soc.*, 1949, **71**, 2226) concerning the configurations of 7-substituents, the trivial indices "α" and "β" employed in the previous papers in this Series (*J.*, 1948, 1783—1803) are now replaced by the true (reversed) indices.

The preparation of the epimeric 7α-hydroxycholest-4-en-3-one was then attempted by similar methods, but Oppenauer oxidation of 7α-hydroxy-, -acetoxy-, and -benzoyloxycholesterol gave products showing only dienone absorption at 2850 Å, due to the presence of cholesta-4 : 6-dien-3-one. However, since 7α-methoxycholest-4-en-3-one can be



(I) (II) (III) (IV)

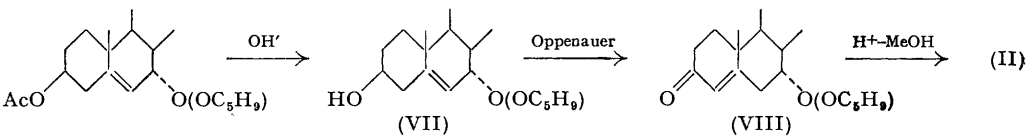
prepared by Oppenauer oxidation of 7α-methoxycholesterol, temporary protection of the 7α-hydroxyl group as an ether was indicated, the ethers (acetals) formed by the addition of 2 : 3-dihydropyran appearing to be most suitable as they can be readily hydrolysed under mild acidic conditions (cf. Greenhalgh, Henbest, and Jones, *J.*, 1951, 1190). The experimental procedure was first investigated with the analogous conversion of 7β-benzoyloxycholesterol into 7-ketocholesterol. Thus the former sterol gave the adduct (V) with dihydropyran, which on alkaline hydrolysis and Oppenauer oxidation gave the unsaturated



(V) (VI)

ketone (VI), the structure of which was confirmed by an alternative method of preparation from 7-ketocholesterol and dihydropyran. Hydrolysis of (VI) by the method employed previously, *viz.*, warm dilute hydrochloric acid in methanol, gave cholesta-3 : 5-dien-7-one, but milder hydrolysis conditions at 20° afforded a good yield of 7-ketocholesterol.

The reaction between dihydropyran and 7α-hydroxycholesteryl acetate (Henbest and Jones, *J.*, 1948, 1792) gave an adduct, which was converted by alkaline hydrolysis into the 3β-sterol (VII). On Oppenauer oxidation, this sterol yielded a non-crystalline ketone



(VII) (VIII)

(VIII), which displayed a single absorption maximum at 2440 Å, as exhibited by Δ^4-3-ketones. Hydrolysis of the protecting pyranyloxy-group under the mild acidic conditions mentioned above for preparing 7-ketocholesterol proceeded more slowly, in accordance with the greater hindrance at (and near) position 7 (cf. the greater ease of hydrolysis of 3-acetates compared with 7-acetates; Henbest and Jones, *loc. cit.*). The only product isolated from the hydrolysis was cholesta-4 : 6-dien-3-one, the 7α-hydroxycholest-4-en-3-one, presumably formed initially, having undergone dehydration in the acidic solution.

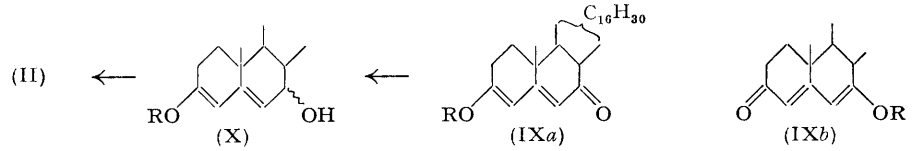
The ease of dehydration of 7α-hydroxycholest-4-en-3-one may well be connected with the fact that the (polar) 7α-hydroxyl group is in the same plane as the (polar) 6β-hydrogen atom, on the assumption that ring B is in the more stable chair form (cf. Barton and Miller, *J. Amer. Chem. Soc.*, 1950, **72**, 1066). In this configuration, therefore, dehydration

should be facilitated, whereas with the 7β-hydroxy-compound, dehydration will be rendered more difficult as the hydroxyl group does not lie in the same plane as either of the hydrogen atoms attached to $C_{(6)}$.

The reduction of " 3 : 7-diketocholestene " (IX*a* and/or *b*; R = H) was investigated as providing a possible route to compounds containing a 7-hydroxy-3-keto-Δ^4-structure. This " diketone," prepared by Barnett, Ryman, and Smith (*J.*, 1946, 526), was shown to exist mainly if not entirely in a monoenolic form, the ready formation of a methyl ether and an acetate being in accordance with this view.

The enol-ketone was prepared as described by Barnett, Ryman, and Smith; however, different physical constants were recorded for some of the compounds described by these authors, and a comparison of the values is given in the Experimental section.

Lithium aluminium hydride reduction of the enol-ketone was first attempted but, under various conditions, non-crystalline products only were obtained. However similar reduction of the methyl ether (IX*a* or IX*b*; R = Me) afforded a 60% yield of cholesta-4 : 6-dien-3-one (II).



The most likely explanation of this result is that the initial methyl ether has structure (IX*a*; R = Me) and not (IX*b*; R = Me), the first product of reduction then being the 7-hydroxy-compound (X) (2 epimers), and that during the working up procedure (with aqueous tartaric acid) anionotropic rearrangement of the hydroxyl group takes place to position 3, whereupon loss of methanol gives the dienone (II). These ideas received confirmation by the results of decomposition of the reduction complex with water instead of tartaric acid. The solution obtained exhibited $\lambda_{max.}$ 2440 Å in agreement with structure (X), and subsequent treatment with aqueous tartaric acid shifted the absorption maximum to that of the dienone at 2850 Å. The ease of rearrangement of the hydroxyl group in (II) parallels the ready rearrangement of the system >C(OH)·CH=CH·OEt to give >C=CH·CHO (Arens and van Dorp, *Rec. Trav. chim.*, 1949, **68**, 604).

The large lævorotations of the enol acetate and methyl ether further support structures corresponding to (IX*a*) and not (IX*b*) for these compounds (see Table, p. 2379). Possible reasons why the compounds adopt the former structure may be related (*a*) to the larger bulk of the 7-methoxy- or 7-acetoxy-group compared with a 7-keto-group, thus causing more hindrance or " overlap " with hydrogen atoms attached to $C_{(15)}$, and (*b*) to stabilization of the system (IX*a*) by a weak hydrogen bond between the 7-carbonyl oxygen and $C_{(15)}$ methylene group (cf. internal constellation of medium-ring ketones; Prelog, *J.*, 1950, 420).

Experimental

In this and the following paper, m. p.s were determined on a Kofler block, rotations were measured in chloroform solutions, and light absorptions were determined in ethanol solutions. The alumina (P. Spence; Grade H) used for chromatography had an activity of between 2 and 3 on the Brockmann–Schodder scale (*Ber.*, 1941, **74**, 75). It was neutralized (and deactivated) by treatment with dilute acetic acid as will be described by Farrar, Hamlet, Henbest, and Jones (forthcoming publication).

Oppenauer Oxidation of 7β-*Hydroxycholesterol*; *Preparation of* 7β-*Hydroxycholest*-4-*en*-3-*one* (III).—A mixture of the above diol (1 g.), dry acetone (15 c.c.), dry benzene (25 c.c.), and aluminium *tert.*-butoxide (6 c.c. of a 25% solution in toluene) was heated under reflux for 4 hours. The steroid was isolated with ether, and the gum obtained was freed from mesityl oxide by the addition of purified xylene followed by evaporation under reduced pressure; this product exhibited light absorption maxima at 2410 and 2845 Å. It was dissolved in light petroleum (b. p. 40—60°) and added to a column of alumina (125 g.), which had been neutralized with aqueous acetic acid (6·25 c.c. of a 10% solution). Development of the chromatogram with light petroleum (b. p. 40—60°)–benzene (2 : 3) afforded a gum (400 mg.), which later solidified to give cholesta-4 : 6-dien-3-one, m. p. and mixed m. p. 79—80°, after recrystallisation from ether–methanol. Development with benzene–ether (9 : 1) gave a product (170 mg.) that solidified in contact with ether and was crude 7β-hydroxycholest-4-en-3-one (see below). Continued

development with the same solvent afforded a gum (130 mg.), followed by 7-ketocholesterol (250 mg.), m. p. and mixed m. p. 169—170°.

The crude 7β-hydroxy-ketone was rechromatographed on neutralized alumina (20 g.). Development with benzene gave a product which after recrystallization from methanol gave 7β-*hydroxycholest*-4-*en*-3-*one* (106 mg.) as needles, m. p. 183·5—184°, $[\alpha]_D^{16}$ +63° (*c*, 0·33) (Found : C, 81·0; H, 11·2. $C_{27}H_{44}O_2$ requires C, 80·95; H, 11·1%). Light absorption : Maxima, 2430 and 3105 Å, ε = 15,500 and 85 respectively.

7β-*Acetoxycholest*-4-*en*-3-*one*.—The 7β-hydroxy-ketone (180 mg.) was acetylated with acetic anhydride (3 c.c.) in pyridine (6 c.c.) at 20° for 24 hours. Recrystallization of the product from light petroleum (b. p. 40—60°) gave the *acetate* (140 mg.) as plates, m. p. 101—102°, $[\alpha]_D^{16}$ +77° (*c*, 0·60) (Found : C, 78·8; H, 10·6. $C_{29}H_{46}O_3$ requires C, 78·65; H, 10·5%). Light absorption : Maxima, 2380 and 3120 Å; ε = 16,100 and 60 respectively.

7β-*Benzoyloxycholest*-4-*en*-3-*one*.—The 7β-hydroxy-ketone (180 mg.) in pyridine (5 c.c.) was treated with benzoyl chloride (2 c.c.) at 0°, the mixture then being kept at 20° for 2 hours. The steroid was isolated with ether; recrystallization of the product from actone–methanol gave the *benzoate* (135 mg.) as needles, m. p. 165—167°, $[\alpha]_D^{16}$ +81° (*c*, 0·53) (Found : C, 80·6; H, 9·55. $C_{34}H_{48}O_3$ requires C, 80·9; H, 9·6%). Light absorption : Maxima, 2350, 2800, and 3135 Å; ε = 27,700, 820, and 70 respectively.

7β-*Benzoyloxy*-3β-*tetrahydro*-2′-*pyranyloxycholesterol* (V).—7β-Benzoyloxycholesterol (1·0 g.), dissolved in pure 2 : 3-dihydropyran (5 c.c.), was treated with phosphorus oxychloride (2 drops), and the mixture was kept at room temperature for 2 hours. The solution was made alkaline with methanolic potassium hydroxide, and the product was precipitated by the addition of aqueous methanol. The *adduct* (1·2 g.) had m. p. 163—165°, $[\alpha]_D^{16}$ +74° (*c*, 0·59) (Found : C, 78·95; H, 10·1. $C_{39}H_{58}O_4$ requires C, 79·25; H, 9·9%). On admixture with starting material the m. p. was depressed to 109—130°.

7β-*Hydroxy*-3β-*tetrahydro*-2′-*pyranyloxycholesterol*.—A solution of potassium hydroxide (1·5 g.) in methanol was added to a solution of the foregoing benzoate (1·0 g.) in hot methanol (30 c.c.) and benzene (7 c.c.), the mixture then being heated under reflux for 4 hours. Water was added to the cooled reaction mixture to precipitate the product. The *adduct* (0·9 g.) had m. p. 136—140°, $[\alpha]_D^{16}$ +1° (*c*, 0·78) (Found : C, 78·85; H, 11·0. $C_{32}H_{54}O_3$ requires C, 78·95; H, 11·2%).

3β-*Tetrahydro*-2′-*pyranyloxycholest*-5-*en*-7-*one* (VI).—(a) *From* 7-*ketocholesterol*. Phosphorus oxychloride (1 drop) was added to a solution of 7-ketocholesterol (1·0 g.) in pure 2 : 3-dihydropyran (5 c.c.), the solution being kept at 20° for 1 hour, some of the product then separating as needles. Isolation with ether gave the *adduct* (1·1 g.)as needles, m. p. 159—164°, $[\alpha]_D$ −90° (*c*, 1·53) (Found : C, 79·2; H, 10·8. $C_{32}H_{52}O_3$ requires C, 79·25; H, 10·8%). Light absorption : Maximum, 2380 Å, ε = 13,500. On admixture with 7-ketocholesterol the m. p. was depressed to 135—140°.

(b) *From* 7β-*hydroxy*-3β-*tetrahydro*-2′-*pyranyloxycholesterol*. A mixture of this compound (150 mg.), dry acetone (2·5 c.c.), dry benzene (4 c.c.), and aluminium *tert.*-butoxide (0·9 c.c. of a 25% solution in toluene) was heated under reflux with the exclusion of moisture for 6 hours. The solid product obtained by isolation with ether was recrystallized from ethanol, to give the adduct (71 mg.), m. p. and mixed m. p. 159—163°.

Hydrolysis of 3β-*Tetrahydro*-2′-*pyranyloxycholest*-5-*en*-7-*one*.—(*a*) A solution of this compound (100 mg.) in ethanol (15 c.c.) containing hydrochloric acid (0·25%) was heated under reflux for 4 minutes. Addition of water and cooling gave cholesta-3 : 5-dien-7-one (65 mg.) as flat needles, m. p. and mixed m. p. 110—113°.

(*b*) A solution of the adduct (50 mg.) in ethanol (5 c.c.) containing hydrochloric acid (0·08%) was kept at 20° for 68 hours. Addition of water gave nearly pure 7-ketocholesterol, m. p. 164—165°, undepressed on admixture with an authentic sample.

Formation and Oppenauer Oxidation of 7α-*Tetrahydro*-2′-*pyranyloxycholesterol* (VII).—Phosphorus oxychloride (2 drops) was added to a solution of 7α-hydroxycholesteryl acetate (200 mg.) in pure 2 : 3-dihydropyran (5 c.c.). After 2 hours at 20° the solution was made alkaline with methanolic potassium hydroxide and the steroid isolated with ether. The resultant gum, dissolved in methanol (12 c.c.), was heated under reflux with potassium hydroxide (0·3 g.), dissolved in methanol (2 c.c.) and water (1 c.c.), for 45 minutes. Isolation with ether, and recrystallization from aqueous methanol gave 7α-tetrahydro-2′-pyranyloxycholesterol (145 mg.) as flat needles, m. p. 137—140°, depressed to 125—139° on admixture with 7α-hydroxycholesterol. This sterol (140 mg.) was oxidized with the same quantities of reagents as described above for 7β-hydroxy-3β-tetrahydro-2′-pyranyloxycholesterol. The resultant product, which exhibited

a single absorption maximum at 2440 Å, was chromatographed on alumina (10 g.), which had been previously neutralized with aqueous acetic acid (0·5 c.c. of a 10% solution). Development with light petroleum (b. p. 40—60°)–benzene (1 : 1) gave a ketonic product (65 mg.) as a gum, and further development with benzene afforded unchanged starting material (63 mg.). The ketonic product (65 mg.) was kept in ethanol (5 c.c.) containing hydrochloric acid (0·08%) at 20° for 6½ days. After the addition of sodium hydrogen carbonate solution, the steroid was isolated with ether. The product, which displayed a single light absorption maximum at 2835 Å, was recrystallized from ether–methanol, to give cholesta-4 : 6-dien-3-one as plates, m. p. and mixed m. p. 81—83°.

Oppenauer Oxidation of 7α-*Hydroxycholesterol.*—A mixture of the diol (200 mg.), aluminium *tert.*-butoxide (400 mg.), acetone (5 c.c.), and benzene (10 c.c.) was heated under reflux for 4 hours. Isolation by the usual procedure gave a gum, which exhibited a single light absorption maximum at 2850 Å, with an intensity corresponding to the presence of 75% of cholesta-4 : 6-dien-3-one. Conversion into the 2 : 4-dinitrophenylhydrazone gave the derivative of cholesta-4 : 6-dien-3-one as dark red plates (from benzene–ethanol), m. p. 233—234°. Light absorption (in chloroform) : Maximum, 4050 Å, ε = 39,000.

Similar oxidations of 7β-acetoxy- and 7α- and 7β-benzoyloxy-cholesterol also afforded cholesta-4 : 6-dien-3-one as the major product (determined spectroscopically), confirmed by preparation of its characteristic 2 : 4-dinitrophenylhydrazone.

" 3 : 7-*Diketocholestene.*"—The following Table lists the physical constants recorded for this compound and certain derived substances. The compounds were prepared by the methods given by Barnett, Ryman, and Smith (*loc. cit.*) except for modifications described later. Light absorption of this compound in ethanol containing a little potassium hydroxide : Maximum, (3925 Å, ε = 62,200 (Barnett *et al.* give : Maximum, 3900 Å, ε = 35,500).

		M. p.	$[\alpha]_D$	$\lambda_{max.}$, Å	$\varepsilon_{max.}$
7-Ketocholesterol	(1) †	160—161°	—	—	—
	(2)	171	−113° (*c*, 2·18)	2370	13,400
				3345	50
5 : 6-Dibromo-7-ketocholesterol	(1)	124—125	−5°	—	—
	(2)	129—129·5	− 15° (*c*, 0·65)	—	—
3 : 7-Diketocholestene	(1)	185—186	−53°	3220	13,500 *
	(2)	184—185	− 49° (*c*, 1·51)	3200	24,300
3-Methoxycholesta-3 : 5-dien-7-one	(1)	136—137	—	3100 *	13,800 *
	(2)	120—121	−323° (*c*, 1·38)	3080	27,600
3-Acetoxycholesta-3 : 5-dien-7-one	(1)	106—108	—	2840	15,200 *
	(2)	115—118	−185° (*c*, 0·74)	2825	22,500
Cholesta-4 : 6-dien-3-one	(2)	80—81	+32°	2850	26,600
Cholesta-3 : 5-dien-7-one	(2)	113—114	−300° (*c*, 2·02)	2770	24,400

(1) Barnett, Ryman, and Smith (*loc. cit.*). (2) This paper.
* Estimated from graphs.
† Bergstrom and Wintersteiner (*J. Biol. Chem.*, 1941, **141**, 597) give m. p. 170—172°, $[\alpha]_D$ − 104°.

7-*Ketocholesterol.*—The following procedure gave a purer product and reduced the volume of the reaction solution to a more convenient amount. A solution of potassium carbonate (9·6 g.) in water (100 c.c.) was added to a solution of 7-ketocholesteryl acetate (30 g.) in ethanol (1·4 l.) and dioxan (300 c.c.), the mixture being shaken for 24 hours. After dilution with water, the steroid was isolated with ether. Recrystallization of the product from benzene–light petroleum (b. p. 30—40°) gave the sterol as needles (20·1 g., 70%) with the above physical constants.

3-*Methoxycholesta*-3 : 5-*dien*-7-*one* (IX*a*; R = Me).—The yield of this compound was raised to 50% by the use of *dry* methanol (Found : C, 81·75; H, 11·0. Calc. for $C_{28}H_{44}O_2$: C, 81·5; H, 10·75%). Treatment of the " 3 : 7-diketocholestene " with excess of diazomethane in ether–methanol at 20° for 1 hour gave only a 25% yield of the methyl ether, much of the original compound being recovered.

3-*Acetoxycholesta*-3 : 5-*dien*-7-*one* (IX*a*; R = Ac).—A solution of 3 : 7-diketocholestene (200 mg.) in pyridine (4 c.c.) and acetic anhydride (2 c.c.) was kept at 20° overnight in an atmosphere of nitrogen. The cooled reaction mixture after dilution with ether at −20° was washed twice with ice-cold dilute hydrochloric acid and then with iced water. The dried (Na_2SO_4) extract was evaporated under reduced pressure and the residue dissolved in methanol. Cooling then yielded an amorphous solid (104 mg.), m. p. 110—116°. Chromatographic purification of this material on neutralized alumina (cf. succeeding experiment) gave the enol *acetate* (80 mg.) as an amorphous solid from methanol (Found : C, 78·75; H, 10·0. $C_{29}H_{44}O_3$ requires C, 79·05; H, 10·05%).

Reduction of 3-*Methoxycholesta*-3 : 5-*dien*-7-*one*.—A solution of lithium aluminium hydride (0·027 g.) in tetrahydrofuran (3 c.c.) was added to the methyl ether (200 mg.) dissolved in tetrahydrofuran (5 c.c.) at 20°. The opalescent solution was stirred for 2 hours at 20° and, after cooling to 0° and addition of an aqueous solution of tartaric acid, the steroid was isolated with ether. The product was dissolved in light petroleum (b. p. 40—60°) and introduced on to alumina (20 g.; neutralized with 1 c.c. of 10% acetic acid). Development of the chromatogram with benzene–light petroleum (b. p. 40—60°) (2 : 3) gave a product (160 mg.) from which cholesta-4 : 6-dien-3-one (110 mg., 60%), having the above physical constants, was obtained by crystallization from methanol–acetone.

Lithium aluminium hydride reduction of " 3 : 7-diketocholestene " was carried out (*a*) with an excess of reducing agent in boiling ether for 3 hours, giving a non-ketonic gum with an absorption maximum at 2400 Å., and (*b*) with an excess of reducing agent in tetrahydrofuran at 20° for 4 hours, giving a ketonic gum, with absorption maxima at 2380 and 2860 Å, from which no crystalline material was isolated by chromatography.

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