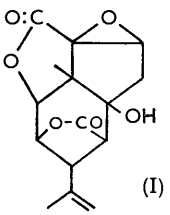
575. *Picrotoxin and Tutin. Part XI.*[1]

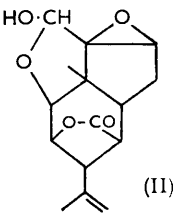
By G. F. Browne, R. B. Johns, and K. R. Markham.

The functional groups of tutin are defined as two five-membered lactones and two hydroxyl groups.

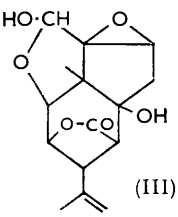
The poisonous constituent of the New Zealand species of *Coriaria*, known collectively [2] as "Tutu," was first isolated by Easterfield and Aston [3] and named tutin. Together with picrotoxinin,[2] $C_{15}H_{16}O_6$, coriamyrtin,[4] $C_{15}H_{18}O_5$, and mellitoxin,[5] $C_{15}H_{18}O_7$, tutin, $C_{15}H_{18}O_6$, forms a series of similar molecular formulæ and physiological properties. Of these bitters, picrotoxinin has been the most intensively studied, efforts culminating in structure (I) being suggested by Conroy.[6] From degradative experiments and Conroy's structure for picrotoxinin, Kariyone and Okuda [7] suggested structure (II) for coriamyrtin. The Japanese workers isolated from *Coriaria japonica* coriamyrtin and a second bitter principle coriarine,[8] which was later shown to be identical with tutin,[9] and because of the similarity of infrared spectra of tutin and picrotoxinin, and the common source of tutin and coriamyrtin, they suggested structure (III) for tutin,[7] *i.e.*, hydroxycoriamyrtin. Structure (III) fails in several respects: a hemiacetal is sufficiently acidic to be methylated with



(I)



(II)



(III)

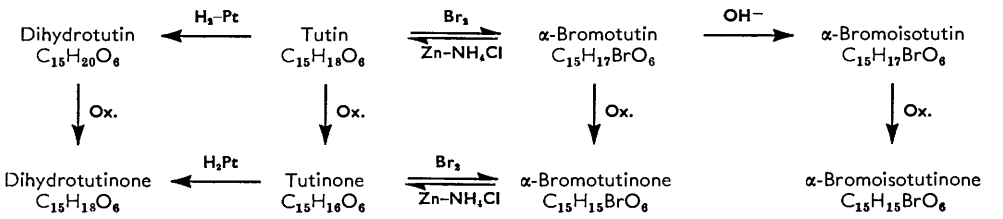
diazomethane, and such a reaction has been observed in the picrotoxinin series [10] in which the lactones are placed similarly to that shown in (III); tutin, however, is recovered from attempted methylation with diazomethane; structure (III) implies that oxidation of tutin would give picrotoxinin but in practice a ketone [11] is formed; picrotoxinin, when heated with alkali, undergoes characteristic degradation; [12] and after slow hydrolysis of the hemiacetal, similar degradation of tutin may be expected since all prerequisites for degradation are present in the molecule; [1] tutin, however, displayed an unsuspected stability to boiling dilute alkali and reacted in a manner most easily explained as the conversion of a dilactone into a dicarboxylic acid. Lastly, a closer examination of the infrared spectra of various tutin derivatives revealed further inconsistencies with the monolactone formulation (III). A re-assessment of the chemistry of tutin was indicated and the results are reported here.

Earlier work has shown tutin to be a polyol, but the number and nature of the hydroxyl groups were not clear from the conflicting reports.[8,9,13] Four different acetates of tutin have been reported and Okuda's [9] attempt to reconcile melting points ranging as widely as

[1] Part X, *J.*, 1960, 1965.
[2] Barth and Kretschy, *Monatsh.*, 1884, **5**, 65.
[3] Easterfield and Aston, *J.*, 1901, **79**, 120.
[4] Riban, *Compt. rend.*, 1866, **67**, 476, 680.
[5] Sutherland and Palmer-Jones, *N.Z. J. Sci. Technol.*, 1947, **29**, *A*, 114.
[6] Conroy, *J. Amer. Chem. Soc.*, 1957, **79**, 1726.
[7] Kariyone and Okuda, *J. Pharm. Soc. Japan*, 1953, **73**, 928.
[8] Kinoshita, *J. Chem. Soc. Japan*, 1930, **51**, 99.
[9] Okuda, *Pharm. Bull. Japan*, 1954, **2**, No. 3, 185.
[10] Johns, Slater, Woods, Brasch, and Gee, *J.*, 1956, 4715.
[11] Fletcher, Hall, Richards, Slater, and Watson, *J.*, 1954, 1953.
[12] Sutter and Schlittler, *Helv. Chim. Acta*, 1947, **30**, 403, 2102; 1949, **32**, 1855, 1860.
[13] Slater, *J.*, 1943, 50.

177° to 240° was unconvincing, particularly when elementary analyses and acetyl determinations were neglected. A re-investigation has shown that tutin is readily acetylated with acetic anhydride and pyridine at water-bath temperature giving a single monoacetate, m. p. 183°. When tutin was refluxed for 1·5 hr. in acetic anhydride with sodium acetate as catalyst, a diacetate, m. p. 203°, was obtained, which presumably corresponds to Okuda's [9] of similar melting point. If tutin monoacetate was further acetylated with sodium acetate as catalyst, a third acetyl derivative, also a diacetate, m. p. 185°, was isolated as the only product. From the published data, it is not possible to determine whether the mono- or di-acetate corresponds with Slater's acetyltutin,[13] m. p. 177°, or Kinoshita's [8] and Okuda's [9] acetate, m. p. 183°. In Part VII [11] it was suggested that tutin possessed at least two hydroxyl groups, and on the basis of a monolactone hypothesis and a Zerewitinoff determination of four active hydrogen atoms, that tutin was possibly a tetrahydroxy-compound. Tutin monoacetate showed hydroxyl absorption in the infrared spectrum which was absent from both the diacetates. The formation of these compounds being assumed to be simple acetylation of the hydroxyl groups, tutin itself is then a dihydroxy-compound.

Further support comes from consideration of the oxidation products. Dihydrotutin, α-bromotutin, and α-bromoisotutin have been oxidised, though in poor yield, to the corresponding ketones by chromic oxide in acetic acid.[11] By oxidation with chromic acid in acetone,[14] nearly quantitative yields of ketones were obtained. In particular, tutin is readily oxidised to tutinone and the double bond (1645 cm.$^{-1}$) is unaffected. (The suffix -one conveniently distinguishes the ketone from the corresponding hydroxy-compound and this nomenclature is adopted in this paper. Thus dehydrotutin [11] becomes tutinone.) Similarly, α-bromotutinone could be readily obtained in high yield from α-bromotutin. The inter-relationships between the hydroxy-compounds and ketones are shown in the scheme below.

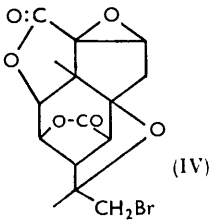


The fact that tutinone can be obtained by oxidation of tutin under both acid and basic (chromic oxide in pyridine) conditions, together with the consistency of elemental analytical data between the series of compounds shown above, suggests that the formation of tutinone is the simple oxidation of a secondary alcohol. There is no fact in the known chemistry of these ketones which is inconsistent with this view. The remaining hydroxyl group is probably tertiary, indicated by the relative difficulty of further acetylation of monoacetyltutin; stability of tutinone to oxidation by chromic acid; and most significantly, the nature of the bromination reaction of tutin.

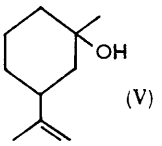
Both tutinone and dihydrotutinone show hydroxyl absorption in the infrared spectrum which in the case of tutinone is removed by bromination to form α-bromotutinone, which is saturated. Similarly, α-bromotutin shows hydroxyl absorption which is absent from its monoacetate. These facts are consistent with the view that tutin is a dihydroxy-compound, and that bromination involves both the double bond and the hydroxy-group, forming a monobrominated derivative. This reaction is parallel to the formation from picrotoxinin (I) of α-bromopicrotoxinin for which structure (IV) has recently been confirmed by crystallographic methods.[15]

[14] Jones, Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2555.
[15] Craven, *Tetrahedron Letters*, 1960, No. 19, 21.

A terminal methylene group in tutin was established by the formation of formaldehyde on ozonolysis [11] but the C_{14} fragment was not isolated. When this reaction was repeated, formaldehyde was again identified and the residue gave a positive



(IV)



(V)

test for a methyl ketone, strongly suggesting the presence of an isopropenyl side chain, hitherto assumed,[7] and which was later confirmed by nuclear magnetic resonance data (see Part XII). It seems reasonable to assume that in tutin a 1,3 relation exists between a suitably reactive hydroxyl group and the isopropenyl side chain as in picrotoxinin. For the reasons outlined above the second hydroxyl group is probably tertiary and is positioned as in the partial formulation (V).

On the basis of the infrared absorption in the carbonyl region of a limited number of derivatives, tutin was considered to be a monolactone.[11] This view was accepted and in turn supported by the Japanese group with their interpretation of the infrared absorption spectra of tutin and α-bromotutin.[16] The resolution of the carbonyl maximum in the spectrum of picrotoxinin is not very pronounced and the resolution has been comparable in our experience in the case of tutin. In particular, for tutin, the lactone absorption maximum, especially for the spectrum in solution, is unsymmetrical with a marked shoulder at higher wave-numbers. With the corresponding ketones, both in solution and as solid, resolution of the lactone absorption band has been marked. The Table lists the maxima in the functional regions, and it should be noted that the lactone-carbonyl

Light-absorption maxima.

	Ultra-violet (mμ)	Infrared (cm.$^{-1}$) Hydroxyl	Lactone	Ketone	Olefin
Picrotoxinin (Nujol)		3464	1790—1766br		1649
Tutin (Nujol)		3510, 3450	1779—1759br		1647
,, ($CHBr_3$)			1789sh, 1776		
Dihydrotutin (Nujol)		3460, 3390	1784, 1756		
,, ($CHBr_3$)			1780, 1771		
Dihydrotutinone (Nujol)		3412	1810—1790br	1705	
,, ($CHBr_3$)			1799sh, 1784		
Tutinone (Nujol)	305	3410	1806, 1794	1705	1645
,, ($CHBr_3$)			1792, 1775	1715	
Ozonolysis product (KBr)	276	3512, 3430	1785, 1769	1695	
α-Bromotutin ($CHBr_3$)			1770		
α-Bromotutinone ($CHBr_3$)	309		1800, 1775	1717	
α-Bromoisotutin (KCl)			1760, 1751br		
α-Bromoisotutinone (Nujol)	314		1780, 1756	1739	

br = broad; sh = shoulder.

absorption maxima of the ketones display marked resolution, consistent with the presence in tutin of a dilactone. The formation of the ketones involved an oxidation step and it may well be argued that acidic conditions have involved simultaneous oxide rupture and oxidation, or other rearrangement in their formation. We rejected this hypothesis on other grounds discussed above, and the spectra of non-oxidised derivatives, *e.g.* dihydrotutin itself, which is resolved in both solution and the solid, as was the ozonolysis product, support this contention. It was also possible to resolve the carbonyl maximum in the infrared spectrum of α-bromoisotutin which is formed by alkaline isomerisation of

[16] Kariyone and Okuda, *Bull. Inst. Chem. Res. Kyoto Univ.*, 1953, **31**, No. 5.

α-bromotutin. The spectroscopic evidence is therefore in complete accord with a dilactone hypothesis.

Support for the monolactone view is found in the results of quantitative titrations of tutin and dihydrotutin,[5,17] which were reported as potentially monobasic under conditions in which picrotoxinin and dihydropicrotoxinin were found to be potentially dibasic. Re-examination of the conductimeric titration curve revealed a reproducible initial uptake of about 1·4 equivalents of cold alkali for tutin, tutinone, and α-bromoisotutin; a titre in excess of experimental error for one equivalent, but which would be consistent with the slow hydrolysis of a second lactone. When the rate of hydrolysis of tutin with 0·1N-sodium hydroxide at 45° was followed, a maximum uptake of 2 equivalents was reached after 11 hours. The same maximum titre was obtained from tutin, tutinone, and α-bromoisotutin by heating them for 15 min. with dilute alkali. These results are consistent with a dilactone structure but it must be noted, in the case of tutin and tutinone, that the reaction is irreversible with either hot or cold alkali, *i.e.* unlike dihydropicrotoxinin or α-bromoisotutin with cold alkali, the tutin derivatives could not be recovered. We had earlier observed that unlike picrotoxinin, tutin could be boiled with alkali, particularly if the solid was first dissolved in the cold alkali, without the development of the degradation colour so characteristic of many picrotoxinin compounds. (If tutin was not previously dissolved, a transient yellow colour could be observed in the solution immediately above the crystals.) If tutin possessed structure (III), degradation should occur.[1] Paper-chromatographic analysis of the alkaline solutions showed the presence of one acidic component only, and both its R_F value and reaction with the developing spray suggested a dibasic nature. Physical methods, then, left little doubt of the potentially dibasic nature of tutin.

Conventional methods which might have yielded carboxylic acid derivatives from tutin by analogy with picrotoxinin, but which in fact resulted in transformations into saturated isomers were summarised in Part VII.[11] Dihydrotutin with hot sodium carbonate has been reported to yield [18] an acid, $C_{15}H_{24}O_8$, and with cold sodium methoxide in methanol an ester,[19] $C_{16}H_{24}O_8$. Little more was known, however of the chemistry of these compounds.

Hydrolysis of tutin with cold dilute aqueous alkali gave no significant yield of crystalline material. Hot dilute aqueous alkali transformed tutin in high yield into a hydrated acid, tutindicarboxylic acid. No evidence has been obtained of the presence in the reaction mixture of any other transformation product. It is formulated as a dicarboxylic acid on the following evidence: Analysis indicates $C_{15}H_{22}O_8$, and the infrared shows absorption in the carbonyl region at 1715—1700 cm.$^{-1}$ only; the double bond is retained; it titrates as a dibasic acid, forms a diphenacyl derivative, and gives a crystalline monomethyl ester with diazomethane, which shows maxima at 1725 (CO_2Me) and 1703 cm.$^{-1}$ (CO_2H).

Methanolysis of picrotoxinin yields dimethyl picrotoxinindicarboxylate and methyl picrotoxate. The ratio of the yields varies according to the concentration of sodium methoxide.[20] With tutin, catalytic quantities of sodium methoxide did not bring about reaction [21] but with one equivalent a good yield of a crystalline ester, $C_{16}H_{24}O_8$, was obtained. This ester contains one carboxyl group (1746, CO_2Me; 1722 cm.$^{-1}$, CO_2H), shows 1·3 acidic centres in titration against cold alkali, and, two after hydrolysis with hot alkali, rapidly decolorises ethereal diazomethane solution, and is unsaturated. It readily forms a triacetate, $C_{22}H_{30}O_{11}$, which still shows hydroxyl absorption in the infrared spectrum. For these reasons we assign it the structure of a monomethyl ester of a dicarboxylic acid rather than that of a monoester monolactone. The mother liquors from the methanolysis yielded an intractable gum which was not further investigated.

[17] Benstead, Brewerton, Fletcher, Martin-Smith, Slater, and Wilson, *J.*, 1952, 1042.
[18] Wilson, M.Sc. Thesis, Victoria Univ. of Wellington, 1950.
[19] Watson, M.Sc. Thesis, Victoria Univ. of Wellington, 1952.
[20] Benstead, Gee, Johns, Martin-Smith, and Slater, *J.*, 1952, 2292.
[21] Fletcher, M.Sc. Thesis, Victoria Univ. of Wellington, 1951.

The dilactone nature of tutin is demonstrated beyond reasonable doubt. It is possible now to assign functions to all six oxygen atoms in tutin as two γ-lactones, and one secondary and one tertiary hydroxyl group. In consequence, the formulation (III) suggested by the Japanese workers for tutin is untenable.

Experimental

Acetylation of Tutin.—(*a*) Tutin (0·5 g.), acetic anhydride (10 ml.), and pyridine (1 ml.) were heated on a steam-bath for 3 hr., then kept overnight at room temperature. After removal of solvent under reduced pressure, the residue was recrystallised from aqueous methanol to give *monoacetyltutin*, m. p. 183° (Found: C, 60·8; H, 5·9; Ac, 13·8. $C_{17}H_{20}O_7$ requires C, 60·7; H, 5·9; 1Ac, 12·8%); $\nu_{max.}$ (potassium bromide disc) 3547m, 3432m, 1781s, 1741s, and 1643w cm.$^{-1}$.

(*b*) Tutin (0·2 g.), sodium acetate (0·05 g.), and acetic anhydride (10 ml.) were heated for 1·5 hr. at 140°. After removal of solvent, the residual oil was washed with water, dissolved in aqueous methanol, and treated with charcoal. The filtrate yielded crystals, which after recrystallisation from aqueous methanol, gave a *diacetyltutin*, m. p. 204° (Found: C, 60·3; H, 5·7; Ac, 22·2. $C_{19}H_{22}O_8$ requires C, 60·3; H, 5·9; 2Ac, 22·2%); $\nu_{max.}$ (in Nujol) 1795s, 1750s, 1732s, and 1650w cm.$^{-1}$.

(*c*) Monoacetyltutin (0·2 g.), sodium acetate (0·05 g.), and acetic anhydride (10 ml.) were refluxed for 1 hr. The solution was worked up as in (*b*) to give a *diacetate*, which crystallised in needles from aqueous methanol, and had m. p. 185° (mixed m. p. with monoacetate was much lower) (Found, in sample sublimed at 156°/0·1 mm.: C, 60·6; H, 5·7; Ac, 22·6%, as required for $C_{19}H_{22}O_8$).

Oxidation of Tutin and Derivatives.—(*a*) Tutin (0·5 g.) was dissolved in acetone (10 ml.) and a solution of chromic acid [14] was added, dropwise, until the mixture remained yellow. The solvent was removed under reduced pressure, the residue was washed with water, and the precipitate was recrystallised from ethanol, yielding tutinone (0·4 g.), m. p. 243° (decomp.); $\lambda_{max.}$ (in ethanol) 305 mμ (log ε 1·22).

In a similar manner the following were oxidised in high yields: dihydrotutin yielded dihydrotutinone, m. p. 245° (decomp.). α-Bromotutin gave α-bromotutinone, m. p. 171°, $\lambda_{max.}$ (in ethanol) 309 mμ (log ε 1·13). α-Bromoisotutin gave α-bromoisotutinone, m. p. 273°.

(*b*) A solution of tutin (0·5 g.) in pyridine (10 ml.) was added to a slurry of chromic oxide (2 g.) and pyridine (20 ml.), and the mixture kept overnight at room temperature. Pyridine was removed in a vacuum and water was added to the residue. Carbon dioxide was bubbled through the mixture, and the buff-coloured precipitate was collected (0·25 g.), recrystallised from ethanol, and treated with charcoal; it yielded tutinone, m. p. and mixed m. p. with an authentic sample, 245°.

Bromination of Tutinone.—A solution of tutinone (0·1 g.) in hot aqueous (15 ml.) dioxan (5 ml.) was treated with bromine. The volume was reduced to *ca.* 10 ml. and on cooling, α-bromotutinone (0·1 g.) crystallised. After recrystallisation from ethanol it had m. p. 165°, undepressed by an authentic sample.

Ozonolysis of Tutin.—Tutin (0·5 g.) was dissolved in acetic acid (25 ml.) and ethyl acetate (3 ml.), and the solution, cooled in an ice bath, was oxidised by a slow stream of ozone and oxygen for 2·5 hr. Water (5 ml.) and zinc dust (1 g.) were added, the suspension was heated on a water-bath for 15 min., excess of zinc filtered off and the filtrate reduced to dryness in a vacuum. The distillate, when treated with 2,4-dinitrophenylhydrazine in 2N-sulphuric acid, yielded a heavy precipitate of formaldehyde 2,4-dinitrophenylhydrazone (m. p. and mixed m. p.). The solid residue was recrystallised from ethyl acetate–ethanol and yielded a slightly impure product, m. p. *ca.* 240°; $\lambda_{max.}$ (in ethanol) 276 mμ; $\nu_{max.}$ (potassium bromide disc) 3545, 3460, 1793s, 1773s, and 1697s cm.$^{-1}$. This material (0·05 g.) was oxidised with potassium iodide (10%; 10 drops) and sodium hypochlorite (20 drops), and the precipitate crystallised from ethanol; it had m. p. 115° undepressed by an authentic sample of iodoform, m. p. 116°.

Tutindicarboxylic Acid.—Tutin (1 g.) was dissolved in 0·1N-aqueous sodium hydroxide (70 ml.) and the solution refluxed for 15 min. The colourless solution was cooled, acidified to pH 2 with concentrated hydrochloric acid, and continuously extracted with ethyl acetate. To the first 24 hours' extract, a few drops of water were added and the whole reduced to a small volume, from which clusters of needles (40% yield) were obtained. Recrystallised from wet

ethyl acetate and dried to constant weight, the *tutindicarboxylic acid* had m. p. 198° (Found: C, 54·4; H, 6·7. $C_{15}H_{22}O_8$ requires C, 54·5; H, 6·7%); $\nu_{max.}$ (in Nujol; hydrated acid, m. p. 98°) 3385 br, 1715—1700, and 1640 cm.$^{-1}$. More (30%) acid was obtained when the aqueous reaction mixture was taken to dryness in a vacuum, excess of hydrochloric acid removed as azeotrope, and the residue crystallised from wet ethyl acetate.

The *monomethyl ester* was prepared by treatment with diazomethane until a yellow colour just persisted. Crystallisation from methanol and ether yielded needles, m. p. 205° (Found: C, 55·7; H, 6·7. $C_{16}H_{24}O_8$ requires C, 55·8; H, 7·0%); $\nu_{max.}$ (potassium bromide disc) 3524, 1725sh, 1703s, and 1640 cm.$^{-1}$. The *p*-bromophenacyl ester was prepared by refluxing for 3 hr. tutin (0·2 g.) in 0·1N-sodium hydroxide (8·6 ml.) and *p*-bromophenacyl bromide (0·5 g.) with sufficient ethanol to ensure solution. When cool, unchanged phenacyl bromide was filtered off and then the *di*-p-*bromophenacyl ester* was obtained; this was recrystallised from aqueous ethanol to constant m. p. 140° (Found: Br, 21·9. $C_{31}H_{32}Br_2O_{10}$ requires Br, 22·1%).

Tutin and Sodium Methoxide.—(The yields in this reaction varied; the following reaction conditions gave the best results.) A solution of sodium methoxide (2 mol.) in dry methanol was added, dropwise, to an ice-cold solution of tutin (1 g.) in dry methanol, and the mixture set aside overnight. Sufficient glacial acetic acid was added to acidify the solution, and the solvent was then removed in a vacuum. The residue was crystallised from aqueous methanol to give amorphous material (0·6 g.) which after further crystallisation gave a *monomethyl ester*, m. p. 220° (Found: C, 56·0; H, 6·6; OMe, 8·7. $C_{16}H_{22}O_8$ requires C, 56·1; H, 6·5; 1OMe, 9·1%); $\nu_{max.}$ (in Nujol) 3530s, 3450s, 3360s, 1746s, 1722s, and 1642w cm.$^{-1}$. The methyl ester was acetylated with acetic anhydride and pyridine giving a *triacetate*, m. p. 84° (with previous softening) (Found, in a sample sublimed at 190°/0·1 mm.: C, 55·9; H, 6·4; Ac, 25·4; active H, 0·48. $C_{22}H_{30}O_{11}$ requires C, 56·2; H, 6·4; 3Ac, 27·4; 2 active H, 0·42%). The methyl ester in water decolorised bromine water and a methanol solution decolorised diazomethane solution.

Conductimeric Titrations.—These were carried out in the usual way: a 20-ml. cell containing platinum electrodes comprised one arm of a Wheatstone bridge and the resistance of a solution, made from a known weight of compound in 0·1N-sodium hydroxide, was determined, as a back-titration was carried out. The potential acidity of the sample was calculated from a plot of conductance against quantity of standard acid.

(*a*) *Tutin.* (i) At room temperature—1·4. (ii) At 45°—after 11 hr. a maximum titre of 2 was attained. (iii) Refluxed for 15 min.—2.

(*b*) *Tutinone.* (i) At 0°—1·48. (ii) 85° for 15 min.—a titre of 2·0.

(*c*) *Tutindicarboxylic acid* (titrated after 35 min.)—2·0.

(*d*) *Tutin + sodium methoxide.* (i) Room temp.—1·35. (ii) After boiling—2·0.

(*e*) *α-Bromoisotutin.* The compound was dissolved in dioxan to ensure solution. (i) At room temp.—1·45 (unchanged material was recovered from this titration). (ii) After boiling—2·0.

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