973. *Lignans. Part II.* Reduction of Lactones of the Podophyllotoxin Group with Lithium Aluminium Hydride.*

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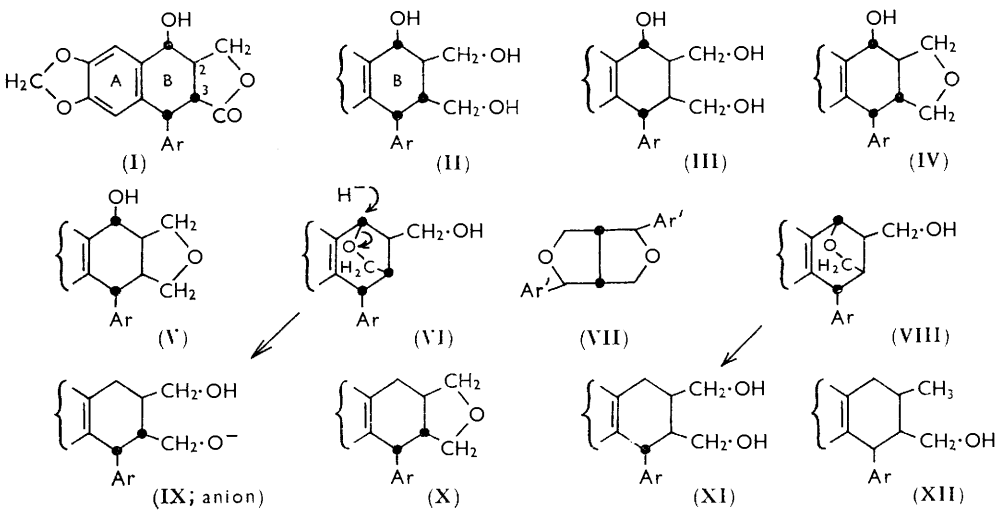
Reduction of podophyllotoxin (I) to a triol having moderate activity against cancer is described. Earlier work on the reduction is reinterpreted in the light of the base-catalysed etherification which may occur under certain conditions, leading to the isolation of anhydropodophyllol (VI) as a typical product. Evidence for the structures assigned to anhydropodophyllol and anhydropicropodophyllol (VIII) is presented.

The optical inactivity of the compound previously isolated [1] on reduction of podophyllotoxin (I) had led us to question [2] the stereospecificity of the reaction on the grounds of possible base-catalysed epimerisation at the labile position 3, with consequent external compensation in a mixture of epimeric triols. This interpretation seemed the more probable when we considered the variety of base-induced changes which may occur during reductions by lithium aluminium hydride, *e.g.*, transesterification [3] and the Lossen rearrangement.[4]



Ar = 3,4,5-$C_6H_2(OMe)_3$; Ar′ = 3,4-CH_2O_2:C_6H_4.

The reduction of podophyllotoxin was therefore repeated with tetrahydrofuran as solvent, so that milder conditions could be employed than were used formerly; [1] in particular, prolonged heating during reduction was avoided. This experiment led to the isolation of an optically active triol, podophyllol (II). In view of previous work [1,5] it seemed possible that dehydration of podophyllol could occur during isolation in an acid medium; however, it was found that pH control was not necessary and that dilute acid could be used with advantage in isolating the triol. When local heating was permitted during the decomposition of the excess of reducing agent a different product was obtained; moreover, it predominated when work was on a small scale. This product, like that obtained by Drake and Price,[1] was optically inactive at the sodium-D line, and

* Part I, Ayres and Denney, *J.*, 1961, 4506.

[1] Drake and Price, *J. Amer. Chem. Soc.*, 1953, **73**, 201.
[2] Ayres and Pauwels, *Proc. Chem. Soc.*, 1961, 388.
[3] Stapp and Rabjohn, *J. Org. Chem.*, 1959, **24**, 1798.
[4] Bauer, *J. Amer. Chem. Soc.*, 1956, **78**, 1945.
[5] Hartwell and Schrecker, *J. Amer. Chem. Soc.*, 1955, **77**, 432, 6725.

active-hydrogen determinations revealed that it was a monohydric alcohol; it can only have been formed by an intramolecular etherification, probably involving attack by alkoxide ion. A clear analogy with this behaviour has been noted [6] during the reduction of moradiol diacetate by lithium aluminium hydride. Optical inactivity in Drake and Price's product could be accounted for by assuming that they had in fact isolated the second product, anhydropodophyllol (IV or VI); this provides a satisfactory alternative explanation to that of external compensation in a mixture of epimers. Dehydration became the only acceptable interpretation once we had observed [2] dextrorotation in anhydropodophyllol at shorter wavelengths, contrasting with lævorotation of podophyllol and of picropodophyllol (obtained by reduction of the epimeric lactone picropodophyllin [1]).

Direct evidence of retention of configuration in podophyllol is afforded by the marked differences in the physical properties of the two triols (podophyllol and picropodophyllol) and of their anhydrides. Anhydropicropodophyllol was obtained having the properties given by Drake and Price, acid-catalysis being needed for the dehydration, which occurred only slowly in xylene; podophyllol, on the other hand, afforded anhydropodophyllol rapidly when heated alone in this solvent. By using less than the theoretical amount of hydride for the reduction it was possible to isolate podophyllol and unchanged podophyllotoxin. If, however, the reaction mixture was allowed to become alkaline during the isolation, picropodophyllin and not podophyllotoxin was recovered; the triol obtained in the same experiment was nevertheless podophyllol, confirming the stereospecificity of the reduction.

The more ready dehydration of podophyllol (II) compared with picropodophyllol (III) would be difficult to account for on the basis of 2,3-cyclisation, to yield (IV) and (V), respectively, since *cis*-2,3- must be preferred to *trans*-2,3-cyclisation as the latter leads to a strained system analogous to that of podophyllotoxin itself. Thus 1,3-cyclisation became the more attractive postulate, particularly as lactones having this type of bridge have been described [7] and a model of anhydropodophyllol (as VI) could be constructed.

It has been shown [8] that mixed benzyl n-butyl ethers are the preferred products of acid-catalysed dehydration of mixtures of butan-1-ol and benzyl alcohol; also the rate of this reaction is increased by electron-donating substituents on the benzene ring, consistent with C–O bond fission in benzyl alcohols. Thus cyclisation of podophyllol to an alkyl–benzyl internal ether (VI) is to be expected; the formation of the necessary intermediate would be enhanced by delocalisation of charge over the aromatic system (this, *per se*, is a further argument for the 1,3-structure), and bond breaking would be assisted by the methylenedioxy-substituent on ring A. An example of acid-catalysed intramolecular etherification of this type is found in the preparation [9] of sesamin (VII). These arguments also indicate that dehydration of picropodophyllol (III) may follow a parallel course, leading to structure (VIII) for anhydropicropodophyllol, which could account for the fact that no olefin-forming dehydration was detected when it was treated with acetic anhydride and sulphuric acid although benzyl alcohols of the " picro " series normally undergo this reaction.[10]

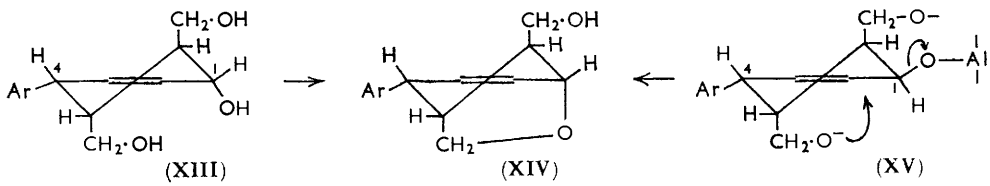
A procedure for the direct reduction of lactones to cyclic ethers by lithium aluminium hydride–boron trifluoride had already been described [11] and appeared to provide an attractive route to one of the possible structures (IV or V); earlier experiments [12] on podophyllotoxin with this reagent were, however, inconclusive. A closely related procedure, with sodium borohydride–boron trifluoride as reagent, has recently [13] been

[6] Barton and Brooks, *J.*, 1951, 257.
[7] Drake and Tuemmler, *J. Amer. Chem. Soc.*, 1955, **77**, 1209; Walker, *ibid.*, 1953, **75**, 3393.
[8] Pratt and Erickson, *J. Amer. Chem. Soc.*, 1956, **78**, 76.
[9] Beroza and Schechter, *J. Amer. Chem. Soc.*, 1956, **78**, 1242.
[10] Hartwell and Schrecker, *J. Amer. Chem. Soc.*, 1952, **74**, 5676, 6321
[11] Pettit and Kasturi, *J. Org. Chem.*, 1960, **25**, 875; 1961, **26**, 4557.
[12] Dr. G. R. Pettit, personal communication.
[13] Ayres, Pauwels, and Pettit, unpublished work.

shown to cause hydrogenolysis of benzyl alcohols and was also applied to anhydropodophyllol. The product was then identified as deoxypodophyllol (IX), which had been previously characterised by Hartwell and Schrecker.[14] The failure to dehydrate this diol to a 2,3-fused tetrahydrofurano-compound under the conditions which were effective for podophyllol is indicative of the formation of a 1,3-fused tetrahydrofuran ring in anhydropodophyllol; the actual isolation of the diol (IX) rather than the anhydride (X) can only be interpreted on the basis of the opening of such a ring (VI ⟶ IX).

The conversion of anhydropicropodophyllol into the diol (XI) by the same reagent confirms its structure (VIII). In these reactions attack by hydride ion is more likely to occur at C-1, although, in an experiment [15] with lithium aluminium hydride–aluminium trichloride, tetrahydrofuran itself underwent fission to butan-1-ol. This could not have affected our results since we did not observe any interaction between the solvent, tetrahydrofuran, and the reagent; moreover, in the event of fission of a 2,3-fused cyclic structure two hydrogenolysis steps must occur, and the rational product would be a monohydric alcohol of type (XII).

Evidence of the mechanism and course of the intramolecular etherification was obtained from a comparison of podophyllol [see (XIII) and epipodophyllol (XV)]. For 1,3-cyclisation these triols must attain the conformation illustrated below; the energy barrier for



(XIII) (XIV) (XV)

lithium aluminium alkoxide

this is probably higher for podophyllol (XIII) since only the aryl group at C-4 has equatorial character whereas in the epimer (XV) the 1-hydroxyl group is also quasi-equatorial. Further, if the mechanism is as formulated above, podophyllol would be expected to react more slowly as a result of broadside attack at C-1 with retention of configuration, rather than the normal rearward attack with Walden inversion characteristic of the epimer and affording anhydropodophyllol (XIV) as the common product. This relative rate of anhydropodophyllol formation was observed in practice. Dehydration of epipodophyllol took place so readily during reduction of epipodophyllotoxin by lithium aluminium hydride that the triol could only be isolated by cooling the reaction mixture strongly during isolation; and, whereas podophyllol was unchanged on melting, epipodophyllol formed its natural anhydride, anhydropodophyllol, when heated. Heating solutions of lithium aluminium alkoxides may prove a useful method of forming cyclic ethers, particularly in the lignan field.

The difference between podophyllotoxin and picropodophyllin was originally ascribed [16] to 1,3-lactonisation in the former and 2,3-lactonisation in the latter; it now appears that competing 1,3-lactonisation may affect the choice of a synthetic route to podophyllotoxin.

Cancer-inhibition by lactones of the podophyllotoxin group has been shown [17] to be restricted to those having the *trans*-2,3-*cis*-3,4 configuration: the 3-epimers are inactive. Tests showed that podophyllol was active against the Walker tumour, as was anhydropodophyllol, although to a smaller extent. Thus a single intraperitoneal injection of 100 mg./kg. led to inhibition; the ratio of control tumour (C)/treated tumour (T) was 2·1 : 1 for podophyllol and 1·24 : 1 for anhydropodophyllol. The toxicity test was negative for podophyllol, and this triol was then applied to the Walker 256 carcinoma, against

[14] Hartwell and Schrecker, *J. Amer. Chem. Soc.*, 1955, **77**, 432, 6725.
[15] Bailey and Marktscheffel, *J. Org. Chem.*, 1960, **25**, 1797.
[16] Borsche and Niemann, *Annalen*, 1932, **499**, 59.
[17] Leiter, Downing, Hartwell, and Shear, *J. Nat. Cancer Inst.*, 1950, **10**, 1273.

which it gave C/T 1·5 after eight daily intraperitoneal injections of 50 mg./kg. It was concluded that the triol was not of therapeutic value, but its inhibitory action provides substantive evidence for the retention of the podophyllotoxin configuration; it also indicates that the activity of these compounds is not entirely dependent on the *trans*-lactone grouping.

A number of acylation procedures have been applied to the alcohols described here, but satisfactory derivatives have not been obtained. There is evidence that structural changes complicate the course of acylation and we hope to publish details later.

Experimental

Infrared spectra were measured for Nujol mulls; those of podophyllol, picropodophyllol, anhydropodophyllol, and anhydropicropodophyllol have been submitted to the D.M.S. scheme.

Preparation of Podophyllol.—Podophyllotoxin (5 g., 0·012 mole) in tetrahydrofuran (50 ml.) was dropped slowly into an ice-cooled, stirred solution of lithium aluminium hydride (3·8 g., 0·1 mole) in tetrahydrofuran (65 ml.). After 4 hr. at 0° the excess of reagent was destroyed by ethyl acetate (22 ml.), and the product was isolated as under (*a*) or (*b*).

(*a*) The reaction mixture was poured into a sodium acetate–hydrochloric acid buffer of pH 1·9 (prepared by adding 150 ml. of N-sodium acetate to 157·5 ml. of N-hydrochloric acid and diluting the mixture to 750 ml.), and the cloudy aqueous solution was extracted with chloroform (4 × 200 ml.); trouble was sometimes experienced with emulsification. The extract was washed with water, dried ($MgSO_4$), and evaporated to a white solid (3·1 g., 61%). The solid isolated from buffers at higher pH was found still to contain lithium.

(*b*) The reaction mixture was poured into 10% hydrochloric acid (500 ml.), and the aqueous solution extracted with chloroform (200 ml., then 3 × 100 ml.). The extracts were washed with water, dried ($MgSO_4$), and evaporated to a white solid (3·3 g., 64%).

Isolation may also be achieved by the use of saturated aqueous ammonium chloride solution [5] but the yields are inferior.

The solid isolated by either method was stirred under alcohol to remove unreduced material and recrystallised from the same solvent, to give colourless crystals of *podophyllol*, m. p. 185—186° (foaming), $[\alpha]_D^{20}$ −183° (*c* 0·08 in $CHCl_3$), $[\alpha]_D^{19}$ −203° (*c* 0·25 in EtOH) (Found: C, 63·0; H, 6·1. $C_{22}H_{26}O_8$ requires C, 63·2; H, 6·3%).

Preparation of Anhydropodophyllol.—Podophyllotoxin (2 g., 0·0048 mole) in tetrahydrofuran (20 ml.) was reduced with lithium aluminium hydride (1·5 g., 0·04 mole) in tetrahydrofuran (26 ml.) as described above. At the end of the reaction the excess of reagent was destroyed by rapid addition of ethyl acetate (9 ml.). Isolation as under (*b*) above yielded a white solid which was stirred under alcohol and recrystallised from the same solvent, yielding colourless *anhydropodophyllol* (1·1 g., 56%), m. p. 253·5—255·5° and $[\alpha]_D^{20}$ 0° (*c* 0·3 in $CHCl_3$) (Found: C, 65·9; H, 6·1. $C_{22}H_{24}O_7$ requires C, 66·0; H, 6·0%).

Preparation of Picropodophyllol.—A suspension of picropodophyllin (2 g., 0·0048 mole) in tetrahydrofuran (100 ml.) was reduced with lithium aluminium hydride (1·5 g., 0·04 mole) in tetrahydrofuran (15 ml.) as described above. The excess of reagent was destroyed with ethyl acetate (9 ml.), and the product isolated as under (*b*) above. Purification was achieved by dissolving the solid in ether (1 l.), concentrating the solution to 250 ml., and keeping it overnight at 0°. In the morning picropodophyllol (1·61 g., 80%) had separated as a white semi-crystalline solid melting over a range (95—108°) and having $[\alpha]_D^{17}$ −70° (*c* 1 in $CHCl_3$); Drake and Price [1] give m. p. 160—162° and $[\alpha]_D$ −67°.

Dehydration of Podophyllol.—Podophyllol (4 g.) was boiled under reflux in xylene (350 ml.) for 30 min. The solution was allowed to cool slowly, whereupon crystals of anhydropodophyllol separated; these were filtered off, washed with xylene and acetone, and recrystallised twice from xylene (yield, 3·1 g., 81%). The anhydropodophyllol obtained by this method had m. p. 240—241° and probably still contained xylene.

Dehydration of Picropodophyllol.—Picropodophyllol (3 g.) was dissolved in boiling xylene (150 ml.), and a small quantity (*ca.* 10 mg.) of toluene-*p*-sulphonyl chloride in xylene (1—2 ml.) was added. The solution was boiled under reflux for 3 hr., then cooled. A large excess of light petroleum (b. p. 80—100°) was added, causing a white precipitate; this was filtered off, washed with light petroleum, and dried in air (yield, 1·8 g., 63%). Attempts to crystallise or otherwise purify this compound were unsuccessful and anhydropicropodophyllol was obtained as an

amorphous white solid having m. p. 73—88° and $[\alpha]_D^{20}$ +79° (*c* 0·8 in $CHCl_3$); Drake and Price [1] give $[\alpha]_D$ +73°.

Reduction of Podophyllotoxin with Less than the Theoretical Amount of Lithium Aluminium Hydride.—Podophyllotoxin (10 g., 0·024 mole) in tetrahydrofuran (100 ml.) was dropped into a solution of lithium aluminium hydride (1 g., 0·026 mole) in tetrahydrofuran (20 ml.) in the usual manner. After 1 hr. a further quantity (2 g.) of podophyllotoxin was added and stirring continued for a second hour. At the end of this time addition of a few drops of ethyl acetate gave no reaction, showing that all the hydride had reacted. Saturated aqueous ammonium chloride solution (50 ml.) was then added [5] with vigorous agitation, and the mixture was allowed to come to room temperature. The insoluble residues were filtered off and washed with boiling alcohol, and the combined washings and filtrate were evaporated under reduced pressure, to give a colourless oil. The oil was immediately taken up in a small quantity of chloroform–tetrahydrofuran (1 : 1), washed with water, dried ($MgSO_4$), and recovered as a white solid (7·8 g.). This was treated with boiling benzene, leading to the separation of two fractions. Fraction A (1·45 g.) was insoluble, and its infrared spectrum was consistent with its being impure podophyllol; after being washed with acetone and recrystallised from alcohol a sample was obtained which was identical with podophyllol. Fraction B (5·72 g.) crystallised from the benzene solution and was identified as podophyllotoxin by its infrared spectrum and rotation {$[\alpha]_D^{23}$ −123°, *c* = 1 in $CHCl_3$)}, and by base-catalysed epimerisation, with piperidine,[18] to picropodophyllin. A similar experiment, in which the mixture was allowed to become alkaline during the isolation, resulted in the isolation of picropodophyllin instead of podophyllotoxin; podophyllol was the other product.

Hydrogenolysis of Anhydropodophyllol.—Anhydropodophyllol (1 g.) and the boron trifluoride–ether complex (15 ml.) in tetrahydrofuran (60 ml.) were dropped into a stirred solution of sodium borohydride (0·85 g.) in tetrahydrofuran (30 ml.) at room temperature. When, after 30 min., no reaction appeared to be taking place the mixture was gently heated to about 50° for an hour. The mixture was then cooled in ice, and the excess of reagent decomposed by the slow addition of hydrochloric acid before chloroform was added, causing separation of two layers; the chloroform layer was run off and the aqueous layer extracted with chloroform. The combined chloroform extracts were washed with sodium hydrogen carbonate solution and water and dried ($MgSO_4$). Evaporation of the solvent gave a pale yellow oil which was seeded with deoxypodophyllol and kept at 0° overnight; solid had then crystallised and an excess of ether was added, leading to the separation of a white solid (0·58 g.). The solid was recrystallised twice from benzene; it then had $[\alpha]_D^{19}$ −156°, m. p. 148—150°, mixed m. p. with deoxypodophyllol 148—150°, and $\nu_{max.}$ 3390 $cm.^{-1}$ (OH).

Treatment of Deoxypodophyllol in Xylene.—Deoxypodophyllol (0·04 g.) was dissolved in cold xylene (10 ml.), and its rotation was determined {$[\alpha]_D^{20\cdot5}$ −141°}. The solution was then boiled under reflux for 30 min., allowed to cool, and the rotation was redetermined {$[\alpha]_D^{21}$ −125°}. The small change in rotation indicated that little dehydration had occurred, whereas under identical conditions podophyllol is completely dehydrated.

Hydrogenolysis of Anhydropicropodophyllol.—Tetrahydrofuran (20 ml.) and boron trifluoride–ether complex (30 ml.) were added to anhydropicropodophyllol (2 g.), giving a deep violet solution which was then dropped slowly into an ice-cooled, stirred solution of sodium borohydride (1·7 g.) in tetrahydrofuran. The colour was discharged immediately on contact with the borohydride, giving a pale yellow solution, which was stirred at 0° for 4 hr. The excess of reagent was decomposed by an excess of hydrochloric acid, and the product was isolated as for deoxypodophyllol above. Evaporation of the chloroform gave an orange oil (1·5 g.), to which methanol (5 ml.) was added, causing the separation of a granular white solid (0·44 g.). This solid was recrystallised twice from methanol–benzene, giving feathery white crystals, m. p. 234—236°, $[\alpha]_D^{21}$ −37° ± 3° (*c* 0·5 in $CHCl_3$), $\nu_{max.}$ 3300b, 3410 $cm.^{-1}$ (OH); Hartwell [14] gives m. p. 237° and $[\alpha]_D$ −32° ± 2° for deoxypicropodophyllol.

Preparation of Epipodophyllol.—Epipodophyllotoxin (1 g., 0·0024 mole) in tetrahydrofuran (10 ml.) was dropped slowly into an ice-cooled, stirred solution of lithium aluminium hydride (0·75 g., 0·02 mole) in tetrahydrofuran (10 ml.). The mixture was stirred at 0° for 4 hr. before being cooled in methanol–carbon dioxide and the excess of reagent was destroyed by very slow addition of ethyl acetate (3 ml.). Saturated ammonium chloride solution (4·5 ml.) was added with vigorous stirring, and, after 30 min., the mixture was allowed to come to room

[18] Robertson and Waters, *J.*, 1933, 83.

temperature. The metallic residues were filtered off and the filtrate was evaporated at 20°/0·5 mm. to a white oil (0·73 g.); this was stirred under water (10 ml.) to remove inorganic salts and then dried *in vacuo*, affording a white solid (0·54 g., 53%). The solid was dissolved in a small volume of methanol at room temperature and set aside; after about 1 week a white solid separated; this was redissolved in cold methanol and set aside. After about 2 months rosettes of colourless *epipodophyllol* separated, having $[\alpha]_D^{20}$ −119° (*c* 0·2 in $CHCl_3$). On the hot stage the crystals lost water at 150—165°, partially melted at 175—180° and finally melted at 249—253° (Found: C, 63·3; H, 6·5. $C_{32}H_{36}O_8$ requires C, 63·2; H, 6·3%).

Reduction of Epipodophyllotoxin: Isolation of Anhydropodophyllol.—Epipodophyllotoxin (1 g., 0·0024 mole) in tetrahydrofuran (10 ml.) was reduced with lithium aluminium hydride (0·75 g., 0·02 mole) in tetrahydrofuran (10 ml.) as previously [1] described for podophyllotoxin. The excess of reagent was decomposed with ethyl acetate (4·5 ml.) after which saturated aqueous ammonium chloride solution (4·5 ml.) was added. The metal-containing residues were filtered off and washed with boiling alcohol, and the combined filtrate and washings were concentrated until a solid began to separate. Overnight the contents of the flask solidified and water was added to remove inorganic salts; the water-insoluble solid was filtered off and washed with alcohol and ether (0·3 g., 31%). After recrystallisation from ethanol it had $[\alpha]_D^{20}$ 0° (*c* 0·26 in $CHCl_3$), m. p. 254—257° and mixed m. p. with anhydropodophyllol 247—255°. The infrared spectrum was identical with that of anhydropodophyllol.

Action of Acetic Anhydride on Anhydropicropodophyllol.—Anhydropicropodophyllol was treated with sulphuric acid in acetic anhydride as described for picropodophyllin.[10] Precipitation by ether followed by chromatography on silica gel led to an amorphous solid having $[\alpha]_D^{17}$ −16° (*c* 0·6 in $CHCl_3$). This product was not fully characterised but it showed no hydroxyl absorption in the infrared whilst in the ultraviolet region $\lambda_{max.}$ was at 291·4 mμ compared with 293·6 mμ for the starting material.

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