Interpretation of the NMR and Circular Dichroic Data of the Sesquiterpene Lactone Thapsigargin

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NMR and CD data of thapsigargin and derivatives confirm the structure suggested by Christensen and Norup and disprove later suggestions.

Results and discussion

The sesquiterpene lactone thapsigargin (**1**) has been isolated and shown to be the major active principle in the umbelliferous plant *Thapsia garganica* L., the resin of which has been used since the antique Greece as a counter-irritant.[1]

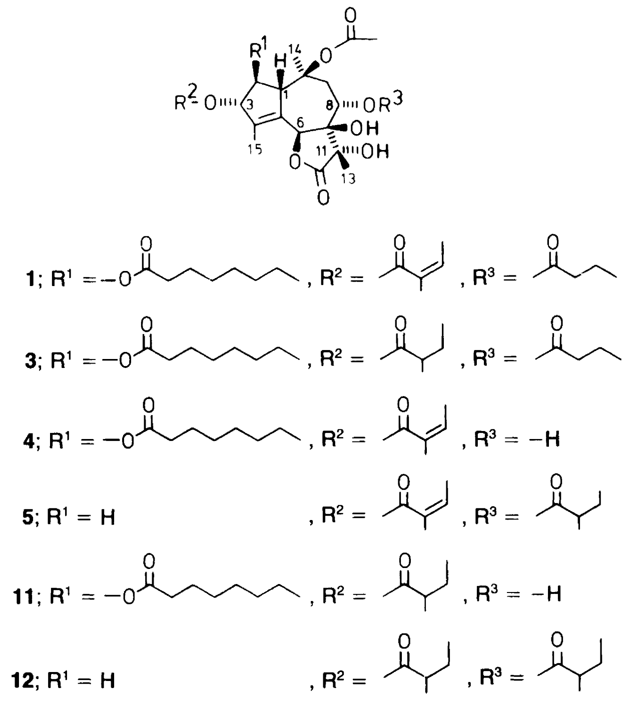
Compound **1** has been shown to induce mediator release from a number of cells involved in the immunological system,[2–5] and to be a tumor promoter in mouse skin.[6] Thapsigargin has recently attracted interest as a model compound for studies of cell activation because of its ability to increase the free cytoplasmic calcium-ion concentration without being an ionophore.[7–9]

The structure assumed for thapsigargin (**1**) was primarily based on an X-ray analysis of the epoxide **2** which was prepared by treatment of **1** with thionyl chloride.[10] The assumption that the stereochemistry is preserved at all the asymmetric centers with the exception of C-7 or C-11 during the conversion of **1** into **2** enabled the relative configuration in thapsigargin to be established save for the two hydroxy-bearing carbons.[10] The assumed stereochemistry at these two centers was based on two arguments: first, the stability of thapsigargin toward periodic acid verifies that the two hydroxy groups are *trans* disposed, and secondly, irradiation of the signal originating from (OH)-7 yields a positive NOE at H-1, which proves that the two hydrogens are juxtaposed.[11] The absolute configuration was based on a comparison of the CD spectra of **1** and the dihydro derivative **3** with the CD spectra of trilobolide (**5**) and the derivative **12**. In both pairs the molar circular dichroism ($\Delta\varepsilon$) of the positive Cotton effect near 215 nm was smaller in the dihydro derivative (Table 1).[12] Thus, the Cotton effect near 215 nm was considered to arise from independent structural features: a positive Cotton effect caused by the 2-methylbutenoyl group esterified with the allylic alcohol, a positive Cotton effect caused by an $n\rightarrow\pi^*$ transition in the lactone carbonyl groups,[13–16] and to a minor extent the Cotton effect of $n\rightarrow\pi^*$ transitions in the remaining carbonyl groups. The big $\Delta\varepsilon$-value of the allylic α,β-unsaturated ester might be explained by assuming an exciton coupling between the 2-methylbutenoyl group and the double bond between C-4 and C-5.[17] Analogous exciton coup-

Table 1. Maxima for the positive Cotton effects in the CD spectra of compounds **1**, **3–5**, and **10–12**.

Compound	λ_{ext}/nm	$\Delta\varepsilon$
1[a]	218	6.1
3[a]	218	2.6
10[b]	215	3.9
4[a]	220	7.3
11[b]	220	4.4
5[a]	214	4.7
12[a]	212	2.2

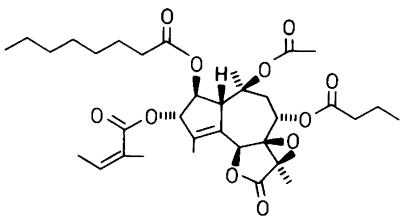
[a]Recorded in methanol solution. [b]Recorded in acetonitrile solution.



lings have been demonstrated in benzoyl esters of allylic alcohols.[18] The smaller Δε-values of the dihydro derivatives **3** and **12** compared with **1** and **5**, respectively, suggested the same absolute configuration at C-3 in **1** and **5**. The relative configuration of trilobolide (**5**) has been established by X-ray crystallography[19] and the absolute configuration was proved by NMR studies on appropriate derivatives.[12]

The above conclusions have recently been questioned by Falsone *et al.*[20] From the roots of *T. garganica* these authors isolated the triester (**4**) along with thapsigargin and the already described thapsigargicin. Comparison of the NMR data published for **4** isolated from the roots[20] with those of compound **4** previously obtained by hydrolysis of **1**,[10] revealed that the two compounds are identical. Falsone *et al.* suggest a revision of the stereochemistry of thapsigargin and the triester on the basis of the following three arguments.[20] First, the easy conversion of the triester into an isopropylidene derivative was taken as a proof of a *cis* disposition of (OH)-7 and (OH)-8. Secondly, the pronounced differences between the ^{1}H NMR spectrum of **1** and that of trilobolide (**5**) led to the suggestion that the stereochemistry at C-1, C-2, C-3 and C-10 was the opposite of that indicated in formula **1**. Thirdly, the positive Cotton effect at 218 nm, which was assumed to originate exclusively in the lactone moiety, led to the conclusion, that the stereochemistry of this moiety is as suggested in formula **1**. Based on these three arguments thapsigargin and the isopropylidene derivative of the triester were assigned the structures **6** and **7**, respectively.[20]

As will be discussed below, the proposed relative configuration of structure **6** offers no explanation for the formation of an epoxide with the relative configuration depicted in formula **2**. The fact that the absolute configuration of the epoxide has never been determined leaves, in principle, the two possibilities that formula **2** represents the correct structure of either the epoxide or the enantiomer. If formula **2** represents the absolute configuration of the epoxide, then the thionyl chloride-promoted conversion of **6** into **2** should involve an inversion at all the chiral centers except for those at C-6 and C-11; an unlikely assumption. The possibility that formula **2** depicts the enantiomer of the epoxide implies that the absolute configuration of **6** and the epoxide agrees at all the asymmetric centers except for C-6



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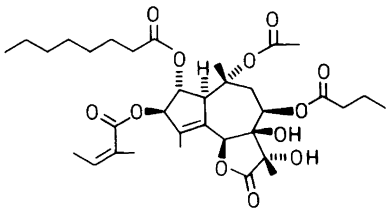

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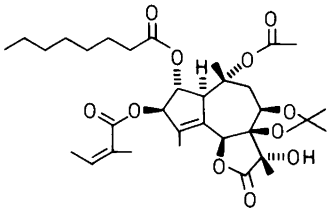

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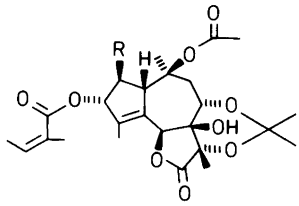
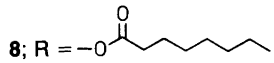
and C-7. The epoxide formation might involve an inversion at C-7, but no obvious explanation can be given for the wrong stereochemistry at C-6. In contrast, formation of the epoxide **2**, as well as an isopropylidene derivative, is consistent with thapsigargin possessing structure **1**. Inspection of the suggested conformation for **1**[11] reveals that O-8 and O-11 are juxtaposed, thus enabling formation of the isopropylidene derivative **8** by treatment of **4** with acetone.

The presence of a 1,3-dioxolane ring in **7** versus a 1,3-dioxane ring in **8** makes the two suggested structures of the isopropylidene derivative distinguishable.[13]C NMR spectroscopic investigations have shown that the chemical shift of the acetal carbon in 2,2-dimethyl-1,3-dioxanes is found in the region 97–101 ppm, whereas the chemical shift of the corresponding carbon in 2,2-dimethyl-1,3-dioxolanes appears between 107 and 112.[21] In addition, the two types of isopropylidene groups may be distinguished by the chemical shifts of the methyl carbons. In dimethyldioxolanes the two methyl groups have very similar chemical shifts, whereas the two signals are separated by approximately 10 ppm in the dimethyldioxanes.[21] In the ^{13}C NMR spectrum of the isopropylidene derivative of **4**, prepared according to the method published by Falsone *et al.*,[20] the signals of the acetal carbon and the two attached methyl carbons appeared at 101.1, 30.4 and 21.1 ppm, respectively, suggesting that the structure of the isopropylidene derivative is **8**. For comparison, the isopropylidene derivative **9** was prepared from trilobolide (**5**). Since **9** is prepared by a reaction between acetone and the triol obtained by the selective hydrolysis of the 2-methylbutanoic ester of **5**, this isopropylidene must possess a 1,3-dioxane ring. In the spectrum of **9**, the signals arising from the acetal carbon and the attached methyl carbons were found at 101.0, 30.5 and 20.8 ppm, respectively.

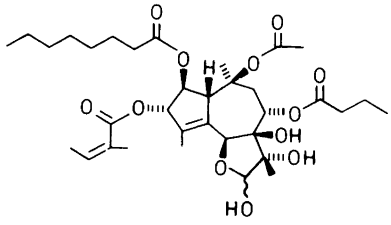
In order to verify the hypothesis that the Cotton effect observed for **1** at 218 nm originates in both the allylic α,β-unsaturated ester and the lactone group, the CD spectrum of the mixture of the lactols **10**[22] was investigated. If the lactone groups is the only group contributing to the Cotton effect of **1** the Δε value of **10** should diminish to zero whereas the Δε value of **3**, in which the





8; R = –O–

9, R = H



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2-methylbutenoyl moiety is reduced, should be comparable to that of **1**. As shown in Table 1, both of these assumptions are invalidated by the experimental findings. In contrast, the observed $\Delta\varepsilon$ values support the hypothesis that the allylic α,β-unsatured ester, as well as the $n\rightarrow\pi^*$ transition of the lactone group, contributes positively to the Cotton effect. If it is assumed that the Cotton effect of the allylic α,β-unsaturated ester, by analogy with the Cotton effect of aromatic allylic esters, originates in an exciton coupling,[18] the absolute configuration at C-3 must be *S* according to the rules for assignment of the stereochemistry.[18] Comparison of the CD-spectra of **5** and **12** by analogy enables the establishment of the *S*-configuration at C-3 in accordance with previously obtained results.[12]

In the case of trilobolide (**5**), application of some empirical rules concerning the relationship between the Cotton effect and the chirality of the lactone ring yields erroneous results. According to Legrand and Bucourt[15] the sign of the Cotton effect of lactones is the opposite of the sign of the torsion angle C–C–C(O)–O. An equivalent rule was suggested by Beecham.[16,23] The torsion angle C(7)–C(11)–C(12)–O(6) of **5** has been determined, by X-ray crystallography,[19,24] to be 27° and consequently a negative Cotton effect originating from the $n\rightarrow\pi^*$ transition of the carbonyl group of the lactone ring would be expected. As shown in Table 1 the CD spectra of **5** and **12** indicate that this Cotton effect is actually positive.

In a paper by Falsone *et al.*,[20] some of the assignments of the signals in the ^{13}C NMR spectrum of **3** have been reversed compared with a previous assignment,[11] and consequently a reassignment of the signals in **1** must be performed. A homonuclear $\{^1H,^1H\}$ correlation diagram (COSY) made possible the unequivocal assignment of all the protons attached to the guaianolide skeleton, except for H-13 and H-14, and a subsequent heteronuclear $\{^1H,^{13}C\}$ correlation diagram (COSY) confirmed the previous assignments[11] of the signals of the carbons bonded to hydrogen.

In conclusion, the above interpretation of the spectra of the isopropylidene derivatives **8** and **9** confirm that thapsigargin and the triester obtained by selective hydrolysis of the butanoic ester of thapsigargin possess the structures **1** and **4**, respectively; thus an additional proposed structure for the triester,[25] should also be rejected.

Experimental

The NMR spectra were recorded on a Bruker AM500 or a Bruker AM250 spectrometer. The standard COSY pulse sequence (magnitude spectra) supplied by Bruker, was employed for the $\{^1H,^1H\}$ correlation. The correlation diagrams were obtained using 90° pulses, 4 scans per trace, 1 s delay between scans, and a spectral window of 2500 Hz to give a 256×204 matrix. The time-domain data were multiplied by an unshifted sine-bell function and the first dimension was zero-filled to 1024 points before the data were transformed into the frequency domain. The diagram was symmetrized after transformation. The standard XH CORR pulse sequence (magnitude spectra), supplied by Bruker, was employed for $\{^1H,^{13}C\}$ correlation. The correlation diagrams were obtained using 90° ^1H pulses and 90° and 180° ^{13}C pulses, 84 scans per trace, 2.0 s delay between scans, and a spectral ^1H window of 750 Hz and ^{13}C window of 12820 Hz to give a 256×8192 matrix. The ^1H dimension was multiplied by an unshifted sine-bell function and zero-filled to 1024, whereas the ^{13}C dimension was multiplied by a Gaussian (GB = 0.5, LB = 0) function before transformation. The diagram was symmetrized after transformation. The experiment was optimized for a 125 Hz coupling constant. Composite pulse decoupling was used to obtain proton decoupling during acquisition. The CD spectra were recorded on a Dichrographe II, Roussel–Jouan.

Chemical-ionisation mass spectra were obtained on a VG 70-70 instrument equipped with a dual EI/CI ion source, using the direct inlet system and isobutane as the reagent gas. The temperature of the ion source was 220 °C.

The isopropylidene derivative (**9**). To a methanolic solution (8 ml) of trilobolide (45 mg, 86 μmol) was added 1 M aqueous sodium carbonate (8 ml) and the mixture was left for 30 min at 0 °C. Hydrochloric acid (2 M, 8 ml) was added to the mixture and the methanol was removed *in vacuo*. The residue was extracted with five portions of ether (10 ml). Concentration of the combined ether phases yielded 44 mg of a gum. The gum was chromatographed by HPLC over LiChrosorp RP 18 (8×250 mm, particle size 5 μm, Knauer) using methanol–water (6:1) as the eluent to give an isomer of the triol obtained by hydrolysis of

the 2-methylbutanoic ester in trilobolide (1 mg), the triol obtained by hydrolysis of the 2-methylbutanoic ester in trilobolide (3 mg), and unchanged trilobolide (28 mg). The crude triol was dissolved in a mixture of acetone (100 µl), 2,2-dimethoxypropane (100 µl), and *p*-toluenesulfonic acid (0.5 mg) and the solution was left for 3 h at room temperature. The solution was concentrated after the addition of 10 drops of triethylamine and the residue was chromatographed over silica gel (Merck 0.06–0.20 mm) using toluene–ethyl acetate (3:1) as the eluent to give 1 mg of **9**. MS [*m/z* (% rel. int.)]: 479 (100, M + 1), 419 (65, M + 1 − acetic acid), 319 (47, M + 1 − acetic acid − angelic acid), 261 (47, M + 1 − acetic acid − angelic acid − acetone). ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.11 (1 H, qq, *J* 7 and 2 Hz, H-3 in 2-methylbutenoyl), 5.86 (1 H, sextet, *J* 2 Hz, H-6), 5.60 (1 H, broad t, ^3J 8 Hz, H-3), 4.28 (1 H, dd, *J* 5 and 3 Hz, H-8), 3.95 (1 H, m, H-1), 2.92 (1 H, dd, *J* 15 and 5 Hz, H-9), 2.58 (1 H, dt, *J* 13 and 8 Hz, H-2), 2.46 (1 H, dd, *J* 15 and 3 Hz, H-9′), 2.02 (3 H, dq, *J* 7 and 2 Hz, β-CH_3 in 2-methylbutenoyl), 1.97 (3 H, s, CH_3 in acetyl), 1.94 (3 H, sextet, *J* 1 Hz, H-15), 1.92 (3 H, quintet, *J* 2 Hz, α-CH_3 in 2-methylbutenoyl), 1.61 (1 H, ddd, *J* 13, 8, and 7 Hz, H-2′), 1.55, 1.54, 1.43 and 1.38 [4×3 H, s, H-13, H-14, $(CH_3)_2$C]. ^{13}C NMR (125 MHz; $CDCl_3$): δ values of the guaianolide nucleus: 172.8 (C-12), 141.4 (C-4), 127.8 (C-5), 85.3 (C-10), 79.2 (C-7), 78.4 (C-3), 78.4 (C-6), 66.1 (C-8), 51.7 (C-1), 38.3 (C-9), 33.3 (C-2), 23.6 (C-14), 15.9 (C-13), 12.7 (C-15); δ values of the isopropylidene group: 101.0 (CH_3–*C*–CH_3), 30.5 (*C*H_3–C–CH_3), 20.8 (CH_3–C–*C*H_3). The δ values of the acyl groups agreed with those previously reported.[26]

The isopropylidene derivative (**8**). The isopropylidene derivative (**8**) was prepared as described for **7**.[20] The ^{1}H NMR data agreed with those published for **7**.[20] MS [*m/z* (% rel. int.)]: 621 (34, M + 1), 561 (25, M + 1 − acetic acid), 521 (100, M + 1 − angelic acid), 461 (72, M + 1 − acetic acid − angelic acid), 417 (75, M + 1 − acetic acid − octanoic acid). ^{13}C NMR (125 MHz; $CDCl_3$): δ values of the guaianolide nucleus: 172.7 (C-12), 139.0 (C-4), 127.4 (C-5), 84.5 (C-10), 83.9 (C-3), 79.2 (C-7), 77.9 (C-2), 77.5 (C-11), 76.0 (C-6), 65.8 (C-8), 57.3 (C-1), 38.0 (C-9), 23.5 (C-14), 15.7 (C-13), 12.5 (C-15); δ values for the isopropylidene carbons: 101.0 (CH_3–*C*–CH_3), 30.4 and 21.1 (*C*H_3–C–*C*H_3). The δ values of the acyl groups agreed with those previously reported.[26]

Compound **11**. To a methanolic solution (3 ml) of **3** (158 mg, 0.24 µmol) was added 0.5 M aqueous sodium carbonate (3 ml) and the mixture was left for 15 min. Hydrochloric acid (4 M, 1.5 ml) was added to the mixture and the methanol was removed *in vacuo*. The residue was extracted with four portions of ether (10 ml). Concentration of the combined ether phases yielded 130 mg of a colourless gum, which was purified by HPLC over LiChrosorp RP 18 (16×250 mm, particle size 10 µm, Knauer) using methanol–water (5:1), to which was added 0.5 % acetic acid as the eluent, to give an isomer of **11** (10 mg), **11** (1 mg), and unchanged **3** (80 mg).

^{1}H NMR data of **11** (250 MHz; $CDCl_3$): δ 5.79 (1 H, broad s, H-6), 5.63 (1 H, broad s, H-3), 5.41 (1 H, t, *J* 2 Hz, H-2), 4.35 (1 H, broad s, H-8), 4.17 (1 H, broad s, H-1), 2.80 (1 H, broad d, *J* 12 Hz, H-9), 2.5–2.0 (4 H, multiplet, H-9, H-2 in octanoyl and 2-methylbutanoyl). 1.89 (3 H, s, acetyl), 1.81 (3 H, broad s, H-15), 1.7–1.5 (10 H, complex signal overlapped by two s at 1.49 and 1.45, H-3 of octanoyl and 2-methylbutanoyl, H-13 and H-14), 1.17 (3 H, d, *J* 7 Hz, CH_3 at C-2 of 2-methylbutanoyl), 0.93 and 0.87 (6 H, two t, H-8 of octanoyl and H-4 of 2-methylbutanoyl). ^{13}C NMR (62.9 MHz; $CDCl_3$): δ values for the guaianolide nucleus: 177.1 (C-12), 141.3 (C-4), 130.6 (C-5), 86.2 (C-10), 84.7 (C-3), 80.1 (C-7 and C-11), 78.6 (C-2), 77.7 (C-6), 69.3 (C-8), 57.9 (C-1), 39.9 (C-9), 23.4 (C-14), 16.9 (C-13), 13.3 (C-15). The δ values of the acyl groups agreed with those previously reported.[26]

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References

1. Rasmussen, U., Christensen, S. B. and Sandberg, F. *Acta Pharm. Suec. 15* (1978) 133.

2. Ali, H., Christensen, S. B., Foreman, J. C., Pearce, F. L., Piotrowsky, W. and Thastrup, O. *Br. J. Pharmacol. 85* (1985) 705.
3. Ohuchi, K., Sugawa, T., Watanabe, M., Hirasawa, N., Tsurufuji, S., Fujiki, H., Sugimura, T., Christensen, S. B. *J. Cancer Res. Clin. Oncol. 113* (1987) 319.
4. Kano, S., Iizuka, T., Ishimura, Y., Fujiki, H. and Sugimura, T. *Biochem. Biophys. Res. Commun. 143* (1987) 672.
5. Mikkelsen, E. O., Thastrup, O. and Christensen, S. B. *Pharmacol. Toxicol. 62* (1988) 7.
6. Hakii, H., Fujiki, H., Suganuma, M., Nakayasu, M., Tahira, T., Sugimura, T., Scheuer, P. J. and Christensen, S. B. *J. Cancer Res. Clin. Oncol. 111* (1986) 177.
7. Thastrup, O., Linnebjerg, H., Bjerrum, P. J., Knudsen, J. B. and Christensen, S. B. *Biochim. Biophys. Acta 927* (1987) 65.
8. Thastrup, O., Foder, B. and Scharff, O. *Biochim. Biophys. Res. Commun. 142* (1987) 654.
9. Altman, J. *Nature (London) 331* (1988) 119.
10. Christensen, S. B., Larsen, I. K., Rasmussen, U. and Christophersen, C. *J. Org. Chem. 47* (1982) 649.
11. Christensen, S. B. and Schaumburg, K. *J. Org. Chem. 48* (1983) 396.
12. Christensen, S. B. and Norup, E. *Tetrahedron Lett. 26* (1985) 107.
13. Jennings, J. P., Klyne, W. and Scopes, P. M. *J. Chem. Soc.* (1965) 7211.
14. Snatzke, G., Ripperger, H., Horstmann, C. and Schreiber, K. *Tetrahedron 22* (1966) 3103.
15. Legrand, M. and Bocourt, R. *Bull. Soc. Chim. Fr.* (1967) 2241.
16. Beecham, A. F. *Tetrahedron Lett.* (1968) 2355.
17. Nakanishi, K. In: Krogsgaard-Larsen, P., Christensen, S. B. and Kofod, H., Eds., *Natural Products and Drug Development,* Munksgaard, Copenhagen 1984, p. 417.
18. Harada, N. and Nakanishi, K. *Circular Dichroic Spectroscopy,* Oxford University Press, Oxford 1983.
19. Kutschabsky, L., Reck, G., Pfeiffer, D. and Ripperger, H. *Z. Chem. 24* (1984) 24.
20. Falsone, G., Haddad, H. and Wendisch, D. *Arch. Pharm. (Weinheim) 319* (1986) 372.
21. Buchanan, J. G., Chacon-Fuertes, M. E., Edgar, A. R., Moorhouse, S. J., Rawson, D. I. and Wightman, R. H. *Tetrahedron Lett. 21* (1980) 1793.
22. Norup, E., Smitt, U. W. and Christensen, S. B. *Planta Med.* (1986) 251.
23. Beecham, A. F. *Tetrahedron Lett.* (1968) 3591.
24. Rychlewska, U. *Personal information.*
25. Holub, M. and Budescinsky, M. *Phytochemistry 25* (1986) 2015.
26. Christensen, S. B., Norup, E., Rasmussen, U. and Madsen, J. Ø. *Phytochemistry 23* (1984) 1659.

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