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Chemical Modification of Ansamitocins. II. Synthesis of 3-Epimaytansionoids *via* 3-Maytansinones

HIROSHI AKIMOTO,* AKIYOSHI KAWAI, NAOTO HASHIMOTO, and HIROAKI NOMURA

Chemistry Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Jusohonmachi, Yodogawa-ku, Osaka 532, Japan

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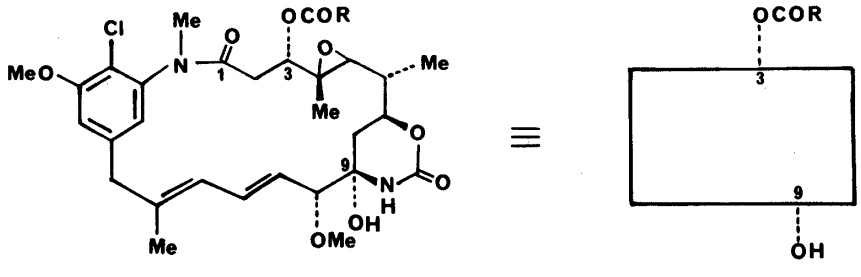
As part of our recent search for new semisynthetic analogs of maytansinoids having a better therapeutic ratio than maytansine, we synthesized 3-epimaytansinoids (VIIIa—c) starting from ansamitocin P-3, a fermentation product of *Nocardia* sp., *via* maytansinol (I). A key intermediate, 3-epimaytansinol (VI), was synthesized by oxidation of I with pyridinium chlorochromate to 3-maytansinone (IV), followed by stereoselective reduction with $NaBH_4$. Esterification of VI with appropriate carboxylic acids gave the corresponding 3-epimaytansinoids (VIIIa—c) in high yields. These compounds did not show the biological activity characteristic of natural maytansinoids to any appreciable degree.

Keywords——ansamitocin; 3-epimaytansinoid; maytansinoid; 3-epimaytansinol; 3-maytansinone; oxidation; sodium boronhydride reduction; stereoselectivity; tubulin polymerization; antitumor activity

The potent antitumor activity and relatively severe side effects of maytansine[1] have prompted us to conduct chemical modification studies of maytansinoids with the aim of developing new semisynthetic analogs with a better therapeutic profile. Maytansinoids, especially those of natural origin, have a 19-membered macrocyclic ring with a conjugated diene (*E, E*), seven chiral centers and a number of functional groups including the C_3 ester side chain, the C_4–C_5 epoxide and the six-membered cyclic carbinolamide moiety[2,3] (Fig. 1). Earlier studies have indicated that the last three functional groups are essential for the antitumor activity.[4,5]

The previous paper[6] from this laboratory reported the synthesis of 4,5-deoxymaytansinoids and demonstrated that their antiprotozoal and antitumor activities are almost the same as those of the corresponding maytansinoids. That finding suggests that the presence of the epoxy group is not essential for the biological activities of the maytansinoids. The next point to be considered is the contribution of the C_3 ester side chain to the antitumor activity. The importance of the presence of this group in the molecule is evident from the fact that



maytansinoid simplified model

Fig. 1

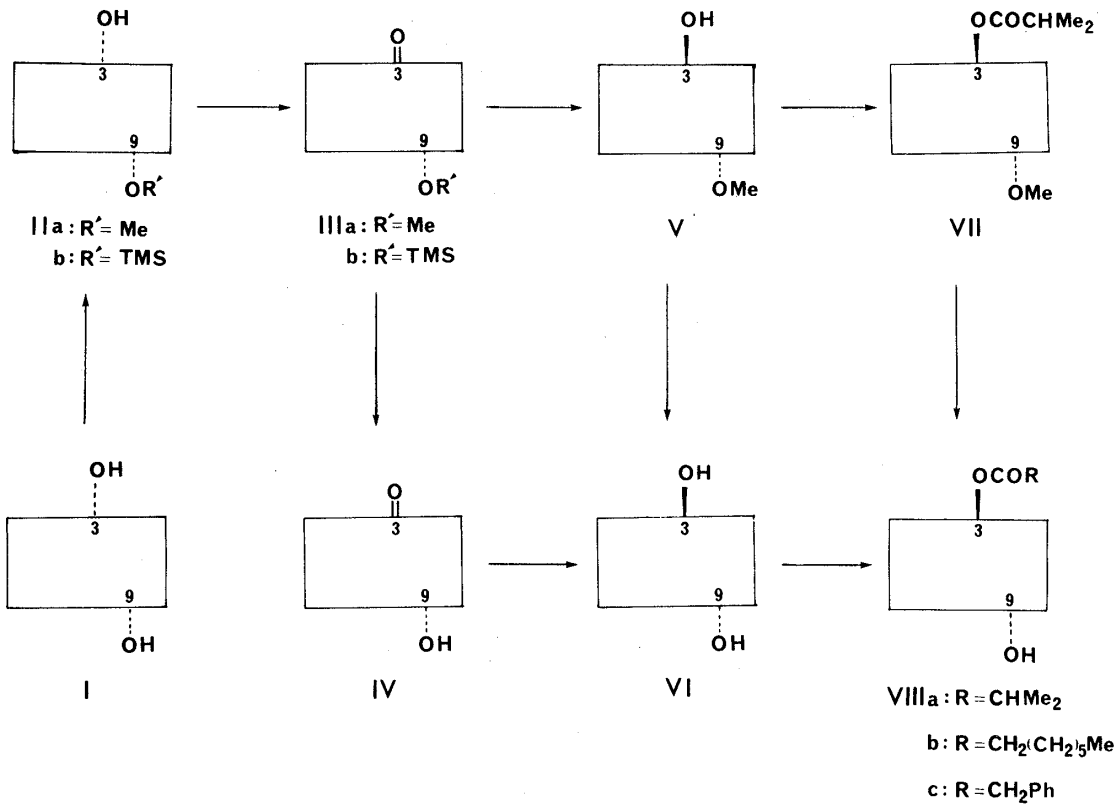


Chart 1

maytansinoids with no ester group at the C_3 position, such as normaysine, maysine and *N*-methylmaysenine, as well as maytansinol, lack the activity.[4] Several modified C_3 esters were synthesized from maytansinol (I) in this laboratory and their structure-activity relationships were partially reported.[7] Changes in the structure of the C_3 ester group of maytansinoids resulted in significant changes in their biological activities. However, the effect of a configurational change at the C_3 ester oxygen on their activity remains to be determined. This report deals with the synthesis and the inhibitory activity of 3-epimaytansinoids, which differ from the maytansinoids in the relative configuration of the hydroxyl or acyloxy group at the C_3 position.

3-Epimaytansinoids (VIII) were synthesized from maytansinol (I) by a process including oxidation of the C_3 hydroxyl to yield 3-maytansinone (IV), followed by reduction to 3-epimaytansinol (VI) and acylation of the C_3 hydroxyl group of VI, as depicted in Chart 1.

The C_9 hydroxyl of I was selectively protected by treating I with methanol in the presence of acid or with *N,O*-bis(trimethylsilyl)acetamide. Subsequently, the reaction of II with pyridinium chlorochromate under neutral conditions gave the C_9-protected C_3 ketone (C_9-protected 3-maytansinone, IIIa, b) which, on deprotection by acid hydrolysis, yielded 3-maytansinone (IV),[8] a key intermediate, in excellent yield. Treatment of maytansinones (IIIa, b and IV) with sodium borohydride gave 3-epimaytansinol (VI) as the main product, although the acid-catalyzed deprotection of the C_9 ether groups was necessary with IIIa, b. The sodium borohydride reduction was repeated under various conditions (Table I); the choice of the starting material, IIIa, b or IV, markedly affected the stereochemistry of the reaction products, while the temperature had only a slight effect. Optimum stereoselectivity was obtained with the unprotected 3-maytansinone (IV). High-performance liquid chromatography (HPLC) revealed that the ratio of the C_3 β-hydroxyl isomer (VI) to I in the reaction products was 9 : 1. The high preference for the C_3 β-hydroxyl isomer over the corresponding

TABLE I. $NaBH_4$ Reduction of 3-Maytansinones

Compounds	Temp. (°C)	VI : I	Yield (%)
IIIa	30	66 : 34	89
IIIa	0	67 : 33	90
IIIa	−20	69 : 31	86
IIIa	−55	71 : 29	85
IIIb	−20	69 : 31	81
IIIb	−55	70 : 30	80
IV	−40	90 : 10	78
IV	−55	90 : 10	77

For the determination of the ratio of 3-epimaytansinol (VI) to maytansinol (I), each sample was analyzed by HPLC (Waters Associates μ-Bondapak C_{18} column, 3.9 mm × 30 cm; 40% aq. CH_3CN, 1 ml/min; detection at 254 nm). The retention times of VI and I were 3.8 and 4.2 min, respectively.

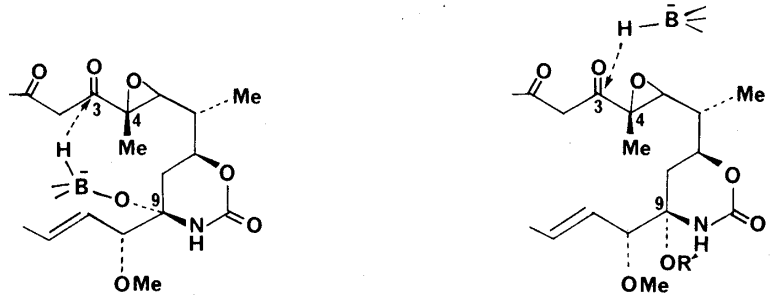


Fig. 2. Possible Transition States of Hydride Reduction

α-isomer (I) suggests that the C_9 α-hydroxyl group of maytansinone (IV) is important in determining the stereochemistry of the product. In accordance with this view, the reduction of IIIa and IIIb with sodium borohydride resulted in the formation of two diastereoisomers in approximately 7 : 3 ratio. Therefore, the reduction of IV is presumed to proceed *via* the formation of the molecular complex between the C_9 α-hydroxyl group and sodium borohydride, followed by intramolecular attack of hydride ion on the carbonyl group from the α-face of the macrocyclic ring, as illustrated in Fig. 2. In contrast, with C_9-protected maytansinones which are unable to form such a complex, the C_3 carbonyl suffers only intermolecular attack by hydride ion, so that the reaction proceeds less stereoselectively, being controlled by a small difference between the α- and β-faces of the molecule in steric hindrance toward the hydride ion.

Esterification of the C_3 hydroxyl group of 3-epimaytansinol (VI) and its C_9 ether (V) was carried out according to the method[8] originally established for the esterification of maytansinol (I). The reaction of these epimaytansinols (VI and V) with appropriate carboxylic acids in the presence of dicyclohexyl carbodiimide (DCCD) and 4-dimethylaminopyridine gave 3-epimaytansinoids (VIIIa—c) in high yields.

The structures of all the semisynthetic epimaytansinoids (V, VI, and VIIIa—c) were readily deduced from the spectral data. 3-Epimaytansinol 9-methyl ether (V) showed an

nuclear magnetic resonance (NMR) spectrum very similar to that of authentic maytansinol 9-methyl ether (IIa), but was characterized by a large downfield shift (0.38 ppm) of the doublet methine proton at the C_5 position (the epoxide ring proton; 2.98 ppm), as compared to the corresponding proton of IIa (2.60 ppm). The mass spectral fragmentation patterns of VI and VIIIa—c were very similar to those of I and the maytansinoids with the corresponding C_3 side chain esters, respectively. The observation of a weak parent peak ion and a characteristically strong ion peak at M^+-61, which corresponds to the loss of H_2O and HNCO from the carbinolamide group, indicated that VI and VIIIa—c have the ansamacrolide ring and molecular weights consistent with the expected structures.

In contrast to the maytansinoids, which have the natural configuration at the C_3 position, these 3-epimaytansinoids (VIIIa—c) showed no appreciable antimicrobial activity against eukaryotic cells and no inhibition of cilian tubulin polymerization in the deciliated protozoan *Tetrahymena pyriformis* W.[9] These results suggest that the epimerization at C_3 in maytansinoids leads to loss of the capability for inhibition of tubulin polymerization, a characteristic biological property of the maytansinoids including maytansine and ansamitocin P-3.

Further chemical modifications of maytansinoids and biological testing are in progress.

Experimental

Melting points were measured on a Yanagimoto MP-S3 hot plate apparatus, and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 215 spectrometer. Mass spectra (MS) were determined with a JMS-01SC spectrometer equipped with a direct inlet system. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained using Varian XL-100-12 and Varian EM-360 instruments: chemical shifts (δ) are reported in ppm downfield from internal TMS. For analytical thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC) pre-coated Kieselgel 60 F_{254} plates (E. Merck, Art. 5642) were used. HPLC was carried out on a Waters ALC/GPC 204 instrument and preparative column chromatography was done on Kieselgel 60 (E. Merck, Art. 7743).

Material——Maytansinol, the starting compound of this study, was prepared by reductive ester cleavage[3] of ansamitocin P-3, a fermentation product of *Nocardia* species.[10]

Maytansinol 9-Methyl Ether (IIa)——A solution of maytansinol (I; 226 mg) and trifluoroacetic acid (0.4 ml) in MeOH (4 ml) was allowed to stand at room temperature for 3 h. The reaction mixture, after neutralization with Na_2CO_3, was poured into ice water and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated *in vacuo*, giving a pale yellow solid. This was chromatographed on silica gel using 2% MeOH in $CHCl_3$ as an eluent. After work-up, IIa (216 mg) was obtained as colorless needles, mp 171—173 °C (ethyl acetate–ether). IR ν_{max}^{KBr} cm^{-1}: 1705, 1640, 1580, 1080. MS *m/e*: 578 (M^+), 502 (M^+-76). NMR (90 MHz, $CDCl_3$) δ: 0.81 (3H, s), 1.23 (3H, d), 1.63 (3H, s), 2.60 (1H, d), 3.17 (3H, s), 3.26 (3H, s), 3.43 (3H, s), 3.94 (3H, s), 5.49 (1H, dd), 6.10 (1H, d), 6.38 (1H, dd), 6.77 (1H, sd), 7.07 (1H, sd).

Maytansinol 9-Trimethylsilyl Ether (IIb)——A mixture of I (195.5 mg), dry triethylamine (0.5 ml), and *N,O*-bis(trimethylsilyl)acetamide (0.22 ml) in dry CH_2Cl_2 (10 ml) was kept at room temperature for 2 h, then evaporated to dryness. The residue was chromatographed on silica gel using 0.8% MeOH in $CHCl_3$ to give the desired product (142.3 mg) as a colorless powder, mp 235—238 °C (dec.) (ethyl acetate–ether). IR ν_{max}^{KBr} cm^{-1}: 1710, 1630, 1580, 1085. MS *m/e*: 636 (M^+).

3-Maytansinone 9-Methyl Ether (IIIa)——A suspension of maytansinol 9-methyl ether (IIa; 214 mg), powdered sodium acetate (1.18 g), and pyridinium chlorochromate (400 mg) in dry CH_2Cl_2 (37 ml) was warmed at 39 °C for 48 h under a nitrogen atmosphere with vigorous stirring. After cooling of the mixture, ethyl ether (74 ml) was added and the resulting inorganic precipitate was filtered off. The filtrate was evaporated *in vacuo* and the residue was chromatographed on silica gel with 0.5% MeOH in $CHCl_3$ to yield IIIa (134 mg) as a pale yellow solid, mp 163—165 °C (ether). IR ν_{max}^{KBr} cm^{-1}: 1730, 1705 sh, 1630, 1575, 1460, 1335, 1090. MS *m/e*: 576 (M^+), 544 (M^+-32), 500 (M^+-76). NMR (90 MHz, $CDCl_3$) δ: 1.02 (3H, s), 1.32 (3H, d), 1.67 (3H, s), 3.22 (3H, s), 3.27 (3H, s), 3.34 (3H, s), 3.96 (3H, s), 4.78 (2H, s).

3-Maytansinone 9-Trimethylsilyl Ether (IIIb)——By means of a procedure similar to that described in the preceding section, IIIb was synthesized from IIb (23.5 mg) as an amorphous powder (13.8 mg). IR ν_{max}^{KBr} cm^{-1}: 1730, 1710, 1635, 1580, 1090. MS *m/e*: 634 (M^+).

3-Maytansinone (IV)——a) From 3-Maytansinone 9-Methyl Ether (IIIa): A solution of IIIa (5.76 mg) in 25% aq. MeOH (1.0 ml) was treated with 2 N HCl (0.2 ml) at room temperature for 2 h. The reaction mixture was neutralized with saturated $NaHCO_3$ and extracted with $CHCl_3$. The combined extracts were dried over $MgSO_4$ and evaporated. Preparative thin-layer chromatography (PTLC) of this residue on Silica gel F-254 plates developed with 2% MeOH in $CHCl_3$ gave a major band (Rf=0.39) which corresponded to that of the desired product. This portion

was collected and extracted with 10% MeOH in $CHCl_3$, giving IV (5.43 mg) as a pale yellow solid, mp 151—152 °C (ethyl acetate–ether). IR ν_{max}^{KBr} cm^{-1}: 1730, 1710, 1630, 1580, 1440, 1335, 1090. MS *m/e*: 562 (M^+), 501 (M^+-61). NMR (90 MHz, $CDCl_3$) δ: 1.02 (3H, s), 1.33 (3H, d), 1.68 (3H, s), 3.20 (3H, s), 3.40 (3H, s), 3.95 (3H, s), 4.80 (2H, s).

b) From 3-Maytansinone 9-Trimethylsilyl Ether (IIIb): In the same manner as described above, treatment of IIIb (6.34 mg) with 2 N HCl gave IV (5.35 mg). HPTLC, HPLC and MS data showed that this compound was identical with the sample obtained from IIIa.

HPLC Analysis of the Products Obtained by $NaBH_4$ Reduction of 3-Maytansinones (IIIa, IIIb and IV)——A solution of 0.01 mmol of 3-maytansinone (IV) or a protected 3-maytansinone (IIIa, b) in MeOH (0.5 ml) was treated with $NaBH_4$ (5.0 mg) for 30 min at the temperature indicated in Table I. The reaction mixture, after being acidified with 2 N HCl, was left standing at room temperature (25 °C) for 2 h, then diluted with water and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*, yielding a diastereomeric mixture of I and IV as a pale yellow solid. To determine the ratio of 3-epimaytansinol (IV) to maytansinol (I), each sample was analyzed by HPLC (μ-Bondapak C-18 column, 3.9 mm × 30 cm, 40% aq. CH_3CN). The results are summarized in Table I.

3-Epimaytansinol 9-Methyl Ether (V)——A solution of IIIa (5.76 mg) in MeOH (0.5 ml) was treated with $NaBH_4$ (5.0 mg) at room temperature for 30 min. After being quenched with 2 N HCl, the mixture was poured into ice water and extracted with $CHCl_3$. The combined organic layer was purified by PTLC on Silica gel F-254 developed with 1.0% MeOH in $CHCl_3$. The silica gel corresponding to the major band was collected and extracted with 1.0% MeOH in $CHCl_3$ to give V (3.45 mg) as a colorless powder, mp 147—149 °C (ether). IR ν_{max}^{KBr} cm^{-1}: 1710, 1655, 1580, 1430, 1085. MS *m/e*: 578 (M^+), 546 (M^+-32), 502 (M^+-76). NMR (90 MHz, $CDCl_3$) δ: 0.72 (3H, s), 1.27 (3H, s), 1.65 (3H, s), 2.98 (1H, d), 3.18 (3H, s), 3.27 (3H, s), 3.40 (3H, s), 3.95 (3H, s), 5.58 (1H, dd), 6.22 (1H, s), 6.37 (1H, s), 6.73 (1H, sd), 6.80 (1H, sd).

3-Epimaytansinol (VI)——A solution of V (5.78 mg) in 50% aq. MeOH (1.0 ml) was treated with 2 N HCl (0.2 ml) at room temperature for 2 h. The reaction mixture was neutralized and extracted with $CHCl_3$. The combined organic layer was evaporated *in vacuo* to yield VI (5.32 mg) as a colorless powder, mp 143—145 °C (ethyl acetate–ether). IR ν_{max}^{KBr} cm^{-1}: 1705, 1650, 1580, 1455, 1430, 1085. MS *m/e*: 564 (M^+), 503 (M^+-61). NMR (90 MHz, $CDCl_3$) δ: 0.75 (3H, s), 1.35 (3H, d), 1.68 (3H, d), 3.22 (3H, s), 3.34 (3H, s), 3.97 (3H, s), 5.60 (1H, dd), 6.75 (1H, sd), 6.78 (1H, sd).

3-Epimaytansinol 9-Methyl Ether 3-Isobutyrate (VII)——A mixed solution of 3-epimaytansinol 9-methyl ether (V; 25 mg), isobutyric acid (37.8 mg), 4-dimethylaminopyridine (10.8 mg), and DCCD (89 mg) in dry CH_2Cl_2 (1.72 ml) was stirred at room temperature for 3 h. The white precipitate that formed was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. Chromatography of the residue on silica gel with $CHCl_3$ as an eluent gave VIIa (27 mg) as a colorless powder. MS *m/e*: 648 (M^+).

3-Epimaytansinol 3-Isobutyrate (VIIIa)——A solution of VII (14 mg) in 50% aq. MeOH (2.2 ml) was treated with 2 N HCl (0.2 ml) at room temperature for 3 h. The reaction mixture was neutralized with saturated $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. Chromatography of the residue on silica gel using 2% MeOH in $CHCl_3$ as an eluent gave VIIIa (13 mg) as colorless needles, mp 145—147 °C (ethyl acetate). IR ν_{max}^{KBr} cm^{-1}: 1730, 1660, 1580, 1460, 1085. MS *m/e*: 634 (M^+), 573 (M^+-61). NMR (90 MHz, $CDCl_3$) δ: 0.67 (3H, s), 1.75 (3H, s), 3.07 (3H, s), 3.37 (3H, s), 3.95 (3H, s), 4.87 (1H, dd), 6.82 (1H, d), 6.98 (1H, d).

3-Epimaytansinol 3-*n*-Octanoate (VIIIb)——In the same manner as described above, 3-epimaytansinol 9-methyl ether (V; 5.78 mg) was esterified by treatment with *n*-octanoic acid in the presence of 4-dimethylaminopyridine and DCCD. Deprotection with 2 N HCl gave VIIIb (4.05 mg) as a colorless powder, mp 116—118 °C (ether). IR ν_{max}^{KBr} cm^{-1}: 1740 sh, 1730, 1710, 1660, 1580, 1460, 1435, 1085. MS *m/e*: 690 (M^+), 629 (M^+-61). NMR (90 MHz, $CDCl_3$) δ: 0.78 (3H, s), 0.87 (3H, s), 1.10—1.80 (13H, m), 1.66 (3H, br s), 3.13 (3H, s), 3.38 (3H, s), 3.97 (3H, s), 6.80 (1H, s), 6.83 (1H, s).

3-Epimaytansinol 3-Phenylacetate (VIIIc)——A mixture of 3-epimaytansinol (VI; 5.64 mg), phenylacetic acid (13.6 mg), 4-dimethylaminopyridine (2.44 mg), and DCCD (20.6 mg) in dry CH_2Cl_2 (0.5 ml) was stirred at room temperature for 30 min. The white precipitate that formed was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. PTLC of the residue on silica gel plates, developed with 2% MeOH in $CHCl_3$, followed by work-up, gave VIIIc (4.32 mg) as colorless prisms, mp 154—156 °C (ethyl acetate–ether). IR ν_{max}^{KBr} cm^{-1}: 1740, 1710, 1660, 1585, 1460, 1085. MS *m/e*: 682 (M^+), 621 (M^+-61). NMR (90 MHz, $CDCl_3$) δ: 0.77 (3H, s), 1.25 (3H, d), 1.68 (3H, br s), 3.15 (3H, s), 3.40 (3H, s), 3.94 (3H, s), 6.68 (1H, s), 6.87 (1H, s), 7.25 (5H, br s).

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