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A NEW SUBGROUP OF BISBENZYLISOQUINOLINE ALKALOIDS: (+)-CYCLEATJEHENINE AND (+)-CYCLEATJEHINE

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Abstract: The leaves of *Cyclea atjehensis* Forman (Menispermaceae) of Thai origin have yielded the bisbenzylisoquinolines (+)-cycleatjehenine (**1a**) and (+)-cycleatjehine (**6**). These alkaloids incorporate the unusual methyleneoxy bridge, and belong to a new bisbenzylisoquinoline subgroup.

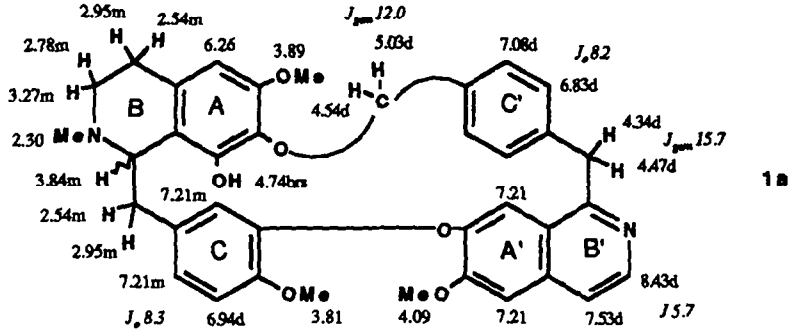
The bisbenzylisoquinolines have been subdivided into more than 15 subgroups, but only the cissampareine subgroup is known to incorporate the unusual methyleneoxy bridge.[3-5] Presently, as a result of the study of *Cyclea atjehensis* Forman (Menispermaceae) of Thai origin, a new subgroup of bisbenzylisoquinolines, consisting of the alkaloids (+)-cycleatjehenine (**1a**) and (+)-cycleatjehine (**6**) , has come to light. This novel subgroup, just like the cissampareine-type alkaloids, is characterized by the presence of a methyleneoxy bridge.

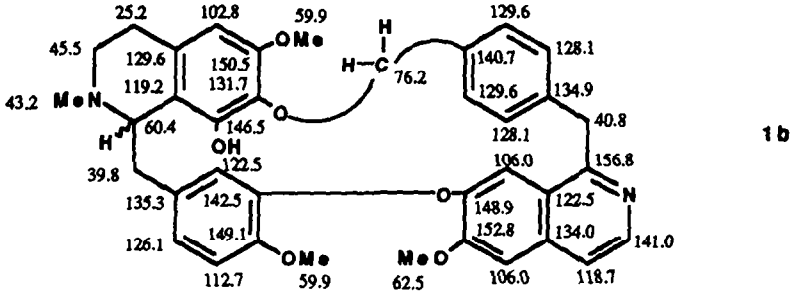
(+)-Cycleatjehenine (**1a**), $C_{37}H_{36}N_2O_6$, is the more prevalent member of this subgroup. The mass spectrum showed molecular ion *m/z* 604 (19%), while the base peak, *m/z* 206, $C_{11}H_{12}NO_3$, corresponded to the tetrahydroisoquinoline moiety represented by rings A and B. The UV spectrum, λ_{max}(MeOH) 238, 279, 314, 327 nm (log ε 4.76, 3.84, 3.41, 3.46), denoted an appreciably conjugated system.

Among the notable features of the 500 MHz ^{1}H NMR spectrum in $CDCl_3$ were an AB system centered at δ 7.53 and 8.43, J_o = *5.7 Hz*, diagnostic of the pyridine ring of an isoquinoline. The α'-methylene group of the benzylisoquinoline moiety was described by two doublets, at δ 4.34 and 4.47 , J_{gem} = *15.7 Hz*. The other half of the dimer consisted of a classical type tetrahydrobenzylisoquinoline, with an N-methyl singlet at δ 2.30 and an H-1 multiplet at δ 3.84. A highly hindered phenolic function was also in evidence, whose proton was responsible for a broad singlet at δ 4.74. Three methoxyl singlets were present, at δ 3.81, 3.89 and 4.09. The downfield shift of the latter methoxyl suggested that it was somehow associated with the isoquinoline moiety. Two doublets relatively downfield at δ 4.54 and 5.03, J_{gem} =*12.0 Hz*, indicated the presence of an extra methylene group flanked by an aromatic ring on one side, and by an oxygen atom on the other. It, therefore, appeared as if a methyleneoxy bridge was present, connecting the two benzylisoquinoline units.

A complete NMR NOE study allowed placement of the three methoxyl substituents at C-6, 12, and 6' (Experimental). Furthermore, irradiation of H-1 (δ 3.84) resulted in enhancements of the N-methyl (δ 2.30), H-10 (δ 7.21) and the two α-protons (δ 2.54 and 2.95). Significantly, no enhancement for an aromatic singlet was in evidence, pointing to substitution at C-8. Another significant NOE data set related one of the α'-protons (δ 4.34) with protons 10' and 14' at δ 6.83. Finally, reciprocating NOE's were also observed between each of the two protons of the methyleneoxy bridge (δ 4.54 and 5.03) on the one hand,

and aromatic protons 11' and 13' (δ 7.08) on the other. It followed that the methylene carbon of the methyleneoxy bridge is probably bonded to C-12'

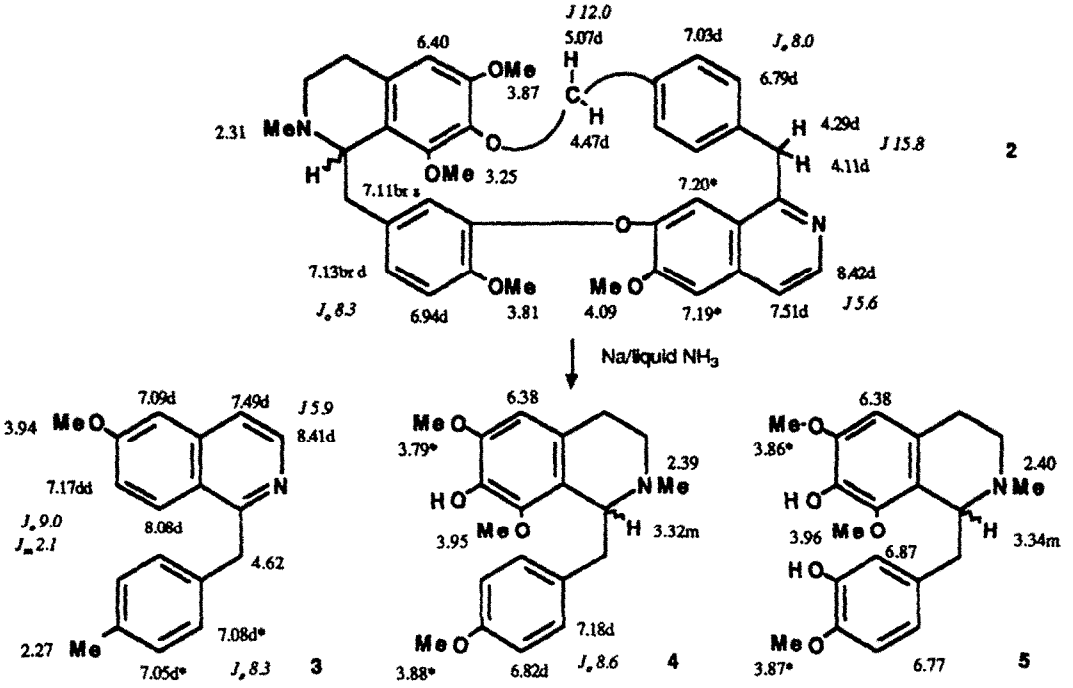




The critical question at this stage was whether the oxygen of the methyleneoxy bridge was linked to C-7 or to C-8. In order to find the answer, a direct ^{1}H-^{13}C correlation was run, supplemented by a long-range ^{1}H-^{13}C coupling study.[6] This allowed us to make a complete ^{13}C NMR chemical shift assignment, which has been indicated in diagram **1b**. Additionally, each of the two protons of the methyleneoxy bridge was coupled to the carbon of that bridge, as well as to C-11' and 13', and also to C-7, thus clearly defining the terminals of the methyleneoxy entity.

Final structure confirmation was provided by a sodium in liquid ammonia reduction of the O-methylated derivative **2**. A complex mixture of products was actually obtained from this reduction, of which three were identified. The first proved to be the optically inactive 6-methoxy-12-methylbenzylisoquinoline (**3**). The second was (-)-N-methyl-6,8,12-trimethoxy-7-hydroxytetrahydrobenzylisoquinoline (**4**), and the third (-)-N-methyl-6,8,12-trimethoxy-7,11-dihydroxytetrahydrobenzylisoquinoline (**5**).

The existence of a methyl group at C-12 in benzylisoquinoline **3** confirmed the attachment of the methyleneoxy carbon to C-12' in (+)-cycleatjehenine (**1**). To determine the position of the phenolic function in the second reduction product **4**, the ^{1}H NMR spectrum was recorded not only in $CDCl_3$ (chemical shift indicated around expression **4**) but also in DMSO-d_6 and then in DMSO-d_6/NaOD. In basic solution, H-5 appeared relatively upfield at δ 6.08, whereas it was at δ 6.45 in DMSO-d_6. This Δδ of 0.37 ppm is indicative of an aromatic proton in a meta relationship to a phenolic hydroxyl.[7] The structure of the third reduction product, **5**, was clarified by means of an NMR NOE study. In particular, reciprocating NOE's were observed between H-10 (δ 6.87) and the 8-methoxyl (δ 3.96).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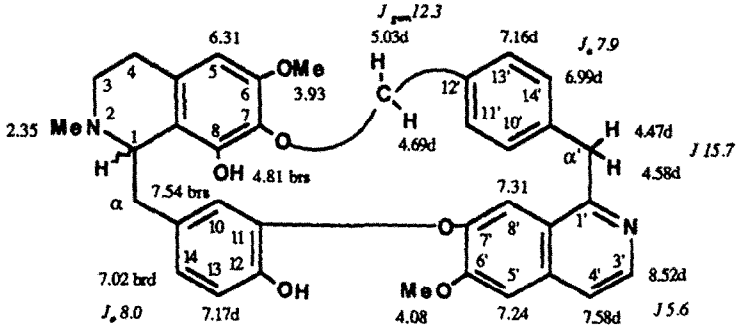
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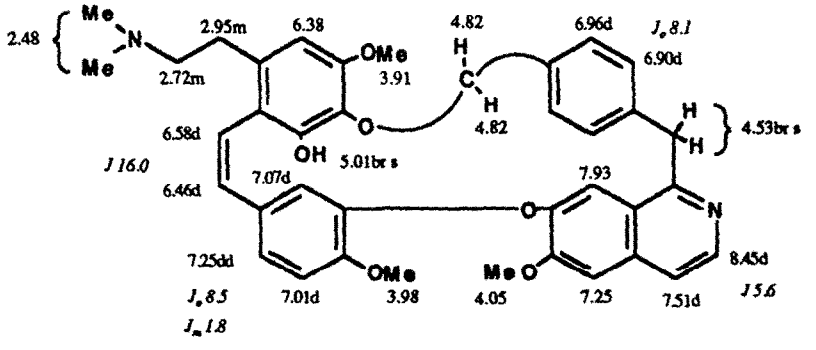
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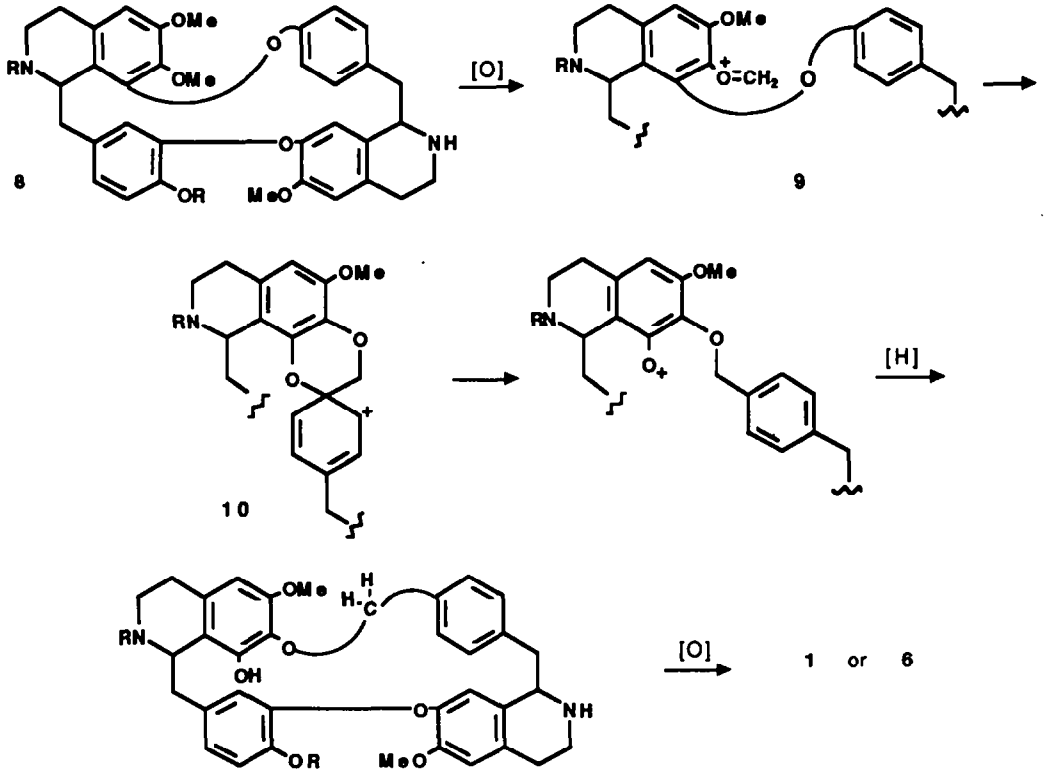
A slightly more polar component from *C. atjehensis* was the diphenolic (+)-cycleatjehine (6), $C_{36}H_{34}N_2O_6$, λ_{max} (MeOH) 237, 280, 314, 327 nm (log ε 4.79, 3.91, 3.46, 3.49). The mass spectrum showed molecular ion *m/z* 590, which is 14 daltons less than for dimer 1. But the base peak, *m/z* 206, representing rings A and B, was identical with that of 1. The ^1H NMR spectrum, which has been summarized around expression 6, bore some resemblance to that of 1, except for the absence of the most upfield methoxyl signal. The H-8' signal now appeared downfield at δ 7.31, while it was at 7.21 in dimer 1

Diazomethane O-methylation of (+)-cycleatjehine (6) furnished three products. The major component, making up 75% of the mixture, proved to be (+)-cycleatjehenine (1). The second product (20%) corresponded to (+)-O-methylcycleatjehenine (2), and the third derivative was the unexpected Hofmann product 7. The spectrum of stilbene 7 showed two N-methyls (δ 2.48) and the characteristic resonances (δ 2.72 and 2.95) of the methylene protons of a dimethylaminoethyl side chain, as well as the two vinylic protons of the stilbenoid bridge (δ 6.46 and 6.58, *J 16.0 Hz*). The ever-present methyleneoxy bridge protons were also in evidence as a broad singlet (δ 4.82).

It is interesting to note that the ratio of 75:20:5 between products 1, 2 and 7 remained essentially constant after about 10 hours under our methylating conditions. A similar situation was encountered when (+)-cycleatjehenine (1) was O-methylated. The starting material 1 was recovered in about 75% yield, while the two products 2 and 7 were obtained in 20% and 5% yields, respectively.

Scheme I



The absolute configuration of bisbenzylisoquinolines is usually established through sodium in liquid ammonia cleavage which furnishes two tetrahydrobenzylisoquinolines whose specific rotations are normaly indicative of their absolute configuration. However, in the present instance, although the sodium in liquid ammonia reduction was duly run, tetrahydrobenzylisoquinolines **4** and **5** that were obtained showed only minimal specific rotations (Experimental), while the CD curves were too weak to be fully significant. This phenomenon is tied to the fact that species **4** and **5** bear a methoxyl substituent at C-8 as well as an N-methyl function. As a consequence, the pendant benzylic ring C lies preferentially in a position nearly perpendicular to the tetrahydroisoquinoline rings A and B, with a resulting lack of distinct optical properties. Presently, therefore, we can only state that the stereochemistry of (+)-cycleatjehenine (**1a**) and (+)-cycleatjehine (**6**) at C-1 is still undetermined.

Given that curine-type alkaloids commonly occur among the Menispermaceae, and are known to be present in *Cyclea* species,[3-5] the biogenesis of (+)-cycleatjehenine (**1**) and (+)-cycleatjehine (**6**) may proceed from a curine analog such as **8** which can suffer oxidation to the oxonium cation **9** (Scheme I). Electrophilic cyclization leads to the dioxane derivative **10**. Cleavage of the dioxane ring as indicated, followed by reduction and oxidation can then furnish species **1** and **6**.

Acknowledgment

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Experimental Section

All NMR data presented were obtained on either a Bruker AM-500 or WM-360 spectrometer in $CDCl_3$. Mass spectral data were collected on a Kratos MS-50 high resolution mass spectrometer.

Plant Collection and Extraction, and Alkaloid Isolation: *Cyclea atjehensis* was collected in Somchitra tin mine, Kanchanaburi Province, near the border with Myanmar, in January 1987. The dried leaves (2 .5 kg) were extracted first with petroleum ether, and then with ethanol. After evaporation of the solvent, the ethanolic extract was mixed with 10% acetic acid in water and filtered. Following basification with NH_4OH, the aqueous solution was extracted with chloroform, which furnished, after evaporation, 55.4 g of crude alkaloids. A rough separation was obtained by silica gel column chromatography using a $CHCl_3$/MeOH gradient. Further purification of the alkaloids was achieved by column chromatography using silica gel for tlc. and through the use of silica gel tlc glass plates. Small, crystalline needles of (+)-cycleatjehenine (**1**)(804 mg) and amorphous (+)-cycleatjehine (225mg) were thus obtained.

(+)-Cycleatjehenine (**1**): $C_{37}H_{36}N_2O_6$; mp 218° (MeOH); EIMS: *m/z* 604 (M+, 19), 590 (3), 598 (8), 574 (3), 399(16), 398 (14), 397 (11), 382 (26), 302 (4), 208 (15), 207 (36), 206 (100), 178 (28); HR MS: for $C_{37}H_{36}N_2O_6$: calc'd 604.2576, fd.604.2543 ; for $C_{26}H_{24}NO_3$: calc'd 398.1749, fd. 398.1764; for $C_{11}H_{24}NO_3$: calc'd 206.0814; fd. 206.0805; UV λ_{max} (MeOH) 238, 279, 314, 327 nm (log ε 4.76, 3.84, 3.41, 3.46); no change in base; λ_{max} (H^+, MeOH) 226sh, 252, 313, 341sh nm; $[\alpha]_D$ +391° (c=0.25, $CHCl_3$), $[\alpha]_D$ +352° (c=0.25, MeOH); CD Δε (nm) 0 (310), -0.6 (285), 0 (270), +105 (244), 0 (232), negative tail below 223 nm.

Principal NOE's: H-5 to OMe-6 (6), OMe-6 to H-5 (10), H-1 to N-Me (2), H-1 to H-10 (5), H-10 to H-1 (5), H-1 to each H-α (1), H-13 to OMe-12 (7), H-5' to OMe-6' (6), OMe-6' to H-5' (9), H-5' to H-4' (6), H-4' to H-5' (2), H-8' to H-α' (δ 4.47) (2), H-α'(δ 4.47) to H-8' (4), H-α' (δ 4.47) to H-10' and H-14' (3), H-10' and H-14' to H-α' (δ 4.47) (2), H-11' and H-13' to CH_2-O (2), CH_2-O to H-11' and H-13' (4).

O-Methylation of (+)-Cycleatjehenine (**1**): In a 10 mL flask, alkaloid **1** (20 mg) was dissolved in MeOH (5 mL), and etheral diazomethane (5 mL) was added. The soln was allowed to stand for 15 h in the refrigerator. Work-up provided **1** (15 mg),

2 (4 mg) and **7** (1 mg). Similar results were obtained when **6** was O-methylated, the products being **1**, **2** and **7** in the identical ratio.

Sodium in liquid ammonia reduction of 2: see reference 8

(+)-O-Methylcycleatjehenine (2) : $C_{38}H_{38}N_2O_6$; EIMS *m/z* 618 (M^+, 10), 604 (4), 603 (9), 399 (2), 398 (3), 382 (6), 309 (6), 221 (26), 220 (100), 206 (15), 204 (13), 203 (14), 192 (11), 190 (4), 178 (21); UV λ_{max} (MeOH) 237, 280, 313, 327 nm (log ε 4.77, 3.95, 3.52, 3.54); no change in base; λ_{max} (H^+, MeOH) 231sh, 252, 283sh, 314, 345sh nm; $[\alpha]_D$ +272°(c=0.11, MeOH), $[\alpha]_D$ +266° (c=0.175, $CHCl_3$).

6-Methoxy-12-methylbenzylisoquinoline (3): $C_{18}H_{17}NO$; EIMS *m/z* 263 (M^+, 38), 262 (100), 247 (13), 204 (13), 162 (62).

(-)-N-Methyl-6,8,12-trimethoxy-7-hydroxytetrahydroisoquinoline (4): $C_{20}H_{25}NO_4$; EIMS m/z 223 (13), 222 (100), 207 (3), 206 (3), 192 (1), 178 (3); [α]D -11° (c=0.64, MeOH), [α]D 0° (c=0.64, CHCl3); CD Δε (nm) 0 (295), -0.5 (283), 0 (260), +0.11 (248), 0 (240), -0.3 (230), positive tail below 220 nm.

(-)-N-Methyl-6,8,12-trimethoxy-7,11-dihydroxytetrahydroisoquinoline (5): $C_{20}H_{25}NO_5$; EIMS *m/z* 358 ($[M-1]^+$, 0.22), 223 (19), 222 (100), 207 (5), 206 (6), 193 (3), 192 (20) 178 (5); $[\alpha]_D$ -12° (c=0.46, MeOH), $[\alpha]_D$ -8° (c=0.46, $CHCl_3$); CD Δε (nm) 0 (298), -0.4 (282), 0 (248), -0.8 (241), 0 (232).

(+)-Cycleatjehine (6): $C_{36}H_{34}N_2O_6$; EIMS *m/z* 590 (M^+, 15), 575 (21), 385 (12), 384 (10), 383 (13), 368 (2), 208 (37), 207 (39), 206 (100), 178 (32); UV λ_{max} (MeOH) 237, 280, 314, 327 nm (log ε 4.79, 3.91, 3.46, 3.49); λ_{max} (OH^-, MeOH) 210, 240, 295, 232 nm; λ_{max} (H^+, MeOH) 226sh, 253, 281sh, 313, 341sh nm; $[\alpha]_D$ +321° (c=0.23, $CHCl_3$), $[\alpha]_D$ +252° (c=0.23, MeOH); CD Δε (nm) 0 (310), -0.5 (286), 0 (270), +97 (241), 0 (232), negative tail below 225 nm.

Stilbene 7: $C_{38}H_{38}N_2O_6$; EIMS *m/z* 618 (M^+, 5), 560 (0.7), 206 (1), 58 (100); UV λ_{max} (MeOH) 221, 237, 294, 328 nm (log ε 4.60, 4.64, 4.05, 3.99); no change in base; λ_{max} (H^+, MeOH) 218, 251, 312, 350sh nm.

Principal NOE's: H-5 to OMe-6 (6), OMe-6 to H-5 (9), H-5 to H-4 (2), H-4 to H-5 (10), H-4 to H-1 (10), H-1 to H-4 (2), H-1 to H-10 (4), H-10 to H-1 (5), H-α to H-10 (3), H-10 to H-α (6), H-α to H-14 (5), H-14 to H-α (6), H-13 to OMe-12 (8), OMe-12 to H-13 (12), H-10 to H-8' (3), H-8' to H-10 (5), H-5' to OMe-6' (5), OMe-6' to H-5' (10), H-5' to H-4' (4), H-4' to H-5' (3), H-8' to H-α' (4), H-α' to H-8' (12), H-α' to H-10' and H-14' (12), H-10' and H-14' to H-α' (4), H-11' and H-13' to CH_2O (3), CH_2O to H-11' and H-13' (16).

References and Notes

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