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LIGNANS FROM *VIROLA AFF. PAVONIS* LEAVES*

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Key Word Index—*Virola aff. pavonis*; Myristicaceae; leaves; lignans; 2,7′-cyclolignans; 7′,8′-*seco*-2,7′-cyclolignan.

Abstract—The petrol fraction of an ethanolic extract from leaves of *Virola aff. pavonis* yielded, beside five known lignans of the aryltetralin, arylnaphthalene and benzoylbenzylbutan types, the four new lignans:, rel-(7*S*,8*S*,8′*R*)-3′, 4′-dimethoxy-3,4-methylenedioxylignan-7-ol, 4-hydroxy-5-methoxy-3′4′-methylenedioxy-2,7′-cyclolignan-7-one, 4-hydroxy-5-methoxy-3′,4′-methylenedioxy-7′,8′-*seco*-2,7′-cyclolignan-7′,8′-dione and 5-methoxy-3′,4′-methylenedioxy-2,7′-cyclolignan-4,7′,8′-triol.

INTRODUCTION

Virola pavonis is a tree called 'Ve-ri-que' by the Karijona Indians from the Colombian Amazon. In previous studies 1,3-diarylpropanoids were isolated from the wood [2], two 3′,7-epoxy-8,4′-oxyneolignans (eusiderins C and D) from the bark [3] and one 7,7′-epoxylignan ((-)-di-de-*O*-methylgrandisidin), one 8,4′-oxyneolignan, one 3′,7-epoxy-8,4′-oxyneolignan (eusiderin E), one phenypropanoid, named pavonisol, from the leaves [4, 5]. Three 3′,7-epoxy-8,4′-oxyneolignan (eusiderins A, C and K), one 8,4′-oxyneolignan type, two 8,3′-neolignans (carinatol and carinatone) and two 4′,7-epoxy-8,3′-neolignans (carinatin and dihydrocarinatin) were isolated from the fruits [6].

The species analysed in the present work was identified as *V. aff. pavonis*. A sample of leaves of this plant was found to contain, besides the known lignans **1a** ((+)-otobaphenol) [7, 8], **2a**, **2b** [9], **3a**, **3b** [10], four new lignans **4**, **5a**, **6** and **7**. Identifications were made from spectral data and absolute configurations were not determined. The nomenclature and numbering of these lignans, although rather cumbersone, follows that outlined by the IUPA-IUB Joint Commission on Biochemical Nomenclature [11].

RESULT AND DISCUSSION

The elemental formula of compound **4**, $C_{21}H_{26}O_5$, was determined by a combination of low-resolution mass spectrometry and NMR counts. The constitutional formula was established by ^1H and ^{13}C NMR which indicated the presence of a 1,4-disubstituted-2,3-dimethylbutan-1-ol with one veratryl and one piperonyl unit. The ^1H NMR spectrum showed the presence of two methyl doublets (δ 0.57 and δ 0.84), two methine groups (δ 1.81, H-8 and δ 2.46, H-8′), one benzylic methylene (δ 2.46 and δ 2.56, 2H-7′) and one benzylic methine group substituted by oxygen (δ 4.31, H-7). The 2D H–H COSY spectrum indicated coupling between the oxygenated methine at δ 4.31 and the methine signal at δ 1.81, which was also coupled to the methyl signal at δ 0.57. On the other hand, the methine signal at δ 2.46 was coupled to the methyl signal at δ 0.84 and the benzylic methylene signal at δ 2.56. These observation are consistent with a lignan-7-olic skeleton. The large low field for H-8′ (δ 2.46) is indicative of intramolecular hydrogen bonding of a hydroxyl group (C-7, OH) with H-8′. The ^{13}C NMR spectrum showed the presence of 3,4-dimethoxyphenyl and 3,4-methylenedioxyphenyl moieties. The veratryl group was not connected to the benzyl alcohol unit because the mass spectrum did not show a fragment ion at *m/z* 167 $[CH(OH)C_6H_3(OMe)_2]^+$; the base peak at *m/z* 151 $[CH(OH)C_6H_3(OCH_2O)]^+$ testifies strongly for the existence of a methylenedioxy benzylalcohol unit. A diastereoisomer of **4**, oleiferina A, was previously isolated from the leaves of *Virola oleifera* [12] but the NMR data and optical rotation are different. The relative configuration of **4** was established by comparison of the ^1H and ^{13}C NMR data, especially in the aliphatic region, with those described for 7,8-*threo*-8,8′-*threo* [10, 12] and 7,8-*threo*-8,8′-*erythro* isomers [13] of lignan-7-ols. Based on this evidence, the structure of **4**

* Part 13 in the series 'The Chemistry of Colombian Myristicaceae'. For Part 12, see ref. [1].

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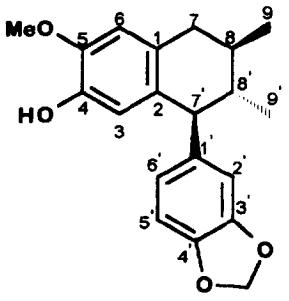

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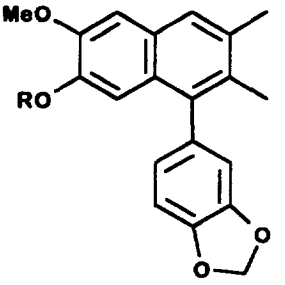

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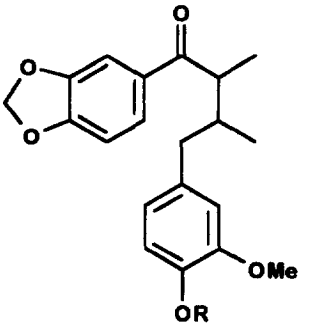

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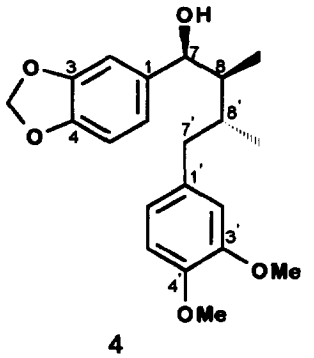

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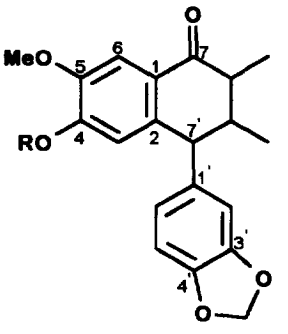

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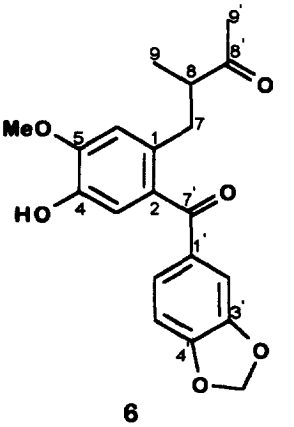

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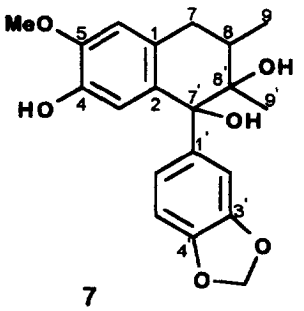

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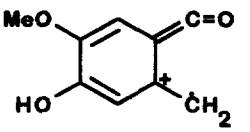

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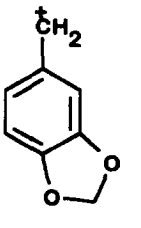

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was established to be rel(7*S*,8*S*,8′*R*)-3′,4′-dimethoxy-3,4-methylenedioxylignan-7-ol.

Compound **5a**, $C_{20}H_{20}O_5$, was recognised as 2,7′-cyclolignan-7-one by joint consideration of the ^{1}H NMR spectrum, which contained a pair of methyl doublets (δ 0.95, δ 1.30), the mass spectrum, which exhibited a prominent $[M-MeCH{=}CHMe]^+$ peak (*m/z* 284) formed by a retro-Diels–Alder cleavage, and the IR spectrum, which showed a conjugated carbonyl absorption part of a benzoyl unit (ν_{max} 1670 cm^{-1}). The position *ortho* to the carbonyl must be unsubstituted, as indicated by the ^{1}H NMR signal for H-6 at low field (δ 7.56). The relative positions of the methylenedioxy and the methoxylhydroxyl groups were determined from mass spectral data which contained peaks at *m/z* 164 (100%) and *m/z* 135 (13%) ascribed to ions **8** and **9**. The position of the hydroxyl was deduced from the ^{1}H NMR spectrum of the acetate **5b**, which did not give a significant change in the signal of H-6 (*s*, δ 7.56 in **5a**; *s*, δ 7.60 in **5b**) but did change the chemical shift of H-3 (*s*, δ 6.28 in **5a**; *s*, δ 6.46 in **5b**). Methylation of **5a** afforded the monomethyl ether with similar ^{1}H NMR data to **5c** reported from *V. sebifera* [14]. Thus, the structure of **5a** was established to be 4-hydroxy-5-methoxy-3′,4′-methylenedioxy-2,7′-cyclolignan-7-one.

Compound **6** had a formula $C_{18}H_{15}O_3(OCH_2O)OMe$ deduced from $[M]^+$ observed in the mass spectrum (*m/z* 356), together with hydrogen, carbon, methylenedioxy and methoxyl counts in the ^{13}C and ^{1}H NMR spectra. In the aliphatic region, the 2D H–H COSY spectrum showed one sextet for a methine (δ 2.95) coupled with both a doublet (δ 1.03, 3H) and a double doublet (δ 2.55, 1H); the latter

proton coupled also with a double doublet (δ 3.05, 1H). These observations could be assigned to the sequence Me-CH-CH_2-Ar. In the ^{1}H NMR spectrum, a singlet (δ 2.03, 3H) of a methyl ketone was observed. Signals at δ 212.7 and 195.9 in the 13C NMR spectrum were assigned to aliphatic and aromatic ketone carbonyls groups, respectively. Other carbon assignments were assisted by a ^{13}C–^{1}H HETCOR experiment. The presence of two aromatic rings was inferred from the ^{13}C NMR spectrum which showed 12 signals between δ 105–155 and in the DEPT experiment five of these signals were CH, in good agreement with the signals of five aromatic protons in the ^{1}H NMR spectrum (δ 6.74, H-6; δ 6.81, H-5′; δ 6.89, H-3; δ 7.30, H-2′, H-6′). In the aromatic region, the 2D COSY H–H spectrum showed only correlation between the multiplet at δ 7.30 and H-5′ (δ 6.81). Thus, it was apparent that one aryl group is trisubstituted and the singlets at δ 6.74 and 6.89 clearly belonged to a tetrasubstituted ring. The methylenedioxy group was located on the trisubstituted ring on the basis of the mass spectrum which showed a peak at *m/z* 149 (39%) [$(OCH_2O)C_6H_3CO$], indicative of the substitution of the carbonyl by piperonyl. The position of methoxyl and hydroxyl groups in the tetrasubstituted ring, were settled through NMR nOe studies. In particular, a nOe was observed between the methoxyl (δ 3.93) and H-6 (δ 6.74). On the basis of these data, the structure of **6** was concluded to be 4-hydroxy-5-methoxy-3′,4′-methylenedioxy-7′,8′-*seco*-2,7′-cyclolignan-7′,8′-dione.

The molecular formula $C_{20}H_{22}O_6$ for compound **7** was determined from the mass spectrum ([M]$^+$, *m/z* 358), ^{1}H and ^{13}C NMR counts. The EI mass spectrum showed peaks at *m/z* 340 [M − 18]$^+$ and 322 [M − 18 − 18]$^+$, which suggested the presence of two alcoholic hydroxyl groups. The compound possessed two aromatic rings, one in the form of a 3,4-dioxyphenyl (^{1}H NMR: *m*, δ 6.7–6.9, 3H) and one in the form of a 3,4-dioxyphenylene-1,2 (2*s*, δ 6.55 and 6.61, 2H). In addition to 12 aromatic carbons, ^{13}C NMR (noise decoupling and DEPT) revealed the presence of a methylenedioxy (δ 100.9), two quaternary carbinolic carbons (δ 80.4, 75.9), one methoxyl (δ 55.9), one methylene (δ 36.7), one methine (δ 34.5) and two methyls (δ 17.3, 15.0). As already suggested by these data, and confirmed by the ^{1}H NMR singlet at δ 0.82 and doublet at δ 1.15, the methyls are linked to one of the carbinolic carbons and at the methine carbon. This was also confirmed by the mass spectrum which showed evidence of a retro-Diels–Alder cleavage in a 2,7′-cyclolignan structure, with loss of 72 mu as MeCHCOHMe to give *m/z* 286. The methoxyl and hydroxyl (^{1}H NMR: *br s*, δ 5.40, exchangeable by D_2O addition) groups, rather than the methylenedioxy group, are located on the tetralinic system on the basis of the mass spectrum which includes a peak at *m/z* 149 (25%) [$(OCH_2O)C_6H_3CO$], indicative of the substitution of the carbinol by piperonyl. These data are in good accordance with structure **7**, a compound which may be the precursor of **6** by oxidative cleavage. Thus, the structure of **7** was established as 3-methoxy-3′,4′-methylenedioxy-2,7′-cyclolignan-4,7′,8′-triol.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded at 90, 200 or 600 MHz and 22.5 or 50 MHz, respectively, in $CDCl_3$ using TMS as int. standard. EIMS were obtained at 70 eV using a direct inlet system. IR spectra were run as neat films, UV spectra in MeOH, except where noted. CC: silica gel (Kieselgel 60, Merck). TLC: silica gel 60 HF_{254}. TLC spots were visualized by UV and exposure of plates to I_2 vapour. Acetates and Me ethers were all prepd by standard methods with Ac_2O and pyridine, and CH_2N_2–Et_2O, respectively.

Plant material. Leaves of *V. aff. pavonis* (A. DC.) A. C. Smith were collected from San Luis region in Magdalena Medio Antioqueño, Colombia, in November 1986 and authenticated by Dr Alvaro Cogollo (Botanic Garden of Medellín). A voucher specimen is deposited in the Herbarium Nacional Colombiano and registered under COL 295037.

Extraction and isolation. Air-dried and powdered leaves (1350 g) were extracted (×4) with EtOH for 2 months at room temp., filtered and the solvent removed to yield a dark green residue (205 g) which was extracted with petrol (54–74°C). The concd extract (19.5 g) was chromatographed on silica gel and elution carried out with solvents of increasing polarity using benzene and EtOAc. A mixt. of lignans was eluted with benzene–EtOAc (9:1, 4:1 and 7:3) and evapn of solvents afforded a residue in each case. These residues were further purified separately by repeated CC and prep. TLC. The residue from benzene–EtOAc (9:1) frs yielded (+)-otobaphenol **1a** (320 mg), **2a** (150 mg) and **2b** (25 mg). Purification of the residues from benzene–EtOAc (4:1 and 7:3) eluates afforded **1a** (110 mg), **2a** (70 mg), **3a** (22 mg), **3b** (26 mg), **4** (28 mg), **5** (25 mg), **6** (20 mg) and **7** (23 mg).

rel-(7*S*,8*S*,8′*R*)-3′,4′-*Dimethoxy*-3,4-*methylene dioxylignan*-7-*ol* (**4**). Viscous oil. $[\alpha]_D$ −57.5° ($CHCl_3$; *c* 0.18). IR ν_{max} cm^{-1}: 3500, 2950, 1600, 1500, 1435, 1240, 1030, 930, 850, 810, 775, 735. UV λ_{max} nm: 230 (log ε 4.563), 282 (log ε 4.279). ^{1}H NMR (600 MHz): δ 0.57 (3H, *d*, *J* = 6.9 Hz, Me-8), 0.84 (3H, *d*, *J* = 6.3 Hz, Me-8′), 1.67 (1H, *br s*, OH-7), 1.81 (1H, *ddq*, *J* = 9.5, 2.4, 6.9 Hz, H-8), 2.46 (2H, *m*, H-7′, H-8′), 2.56 (1H, *dd*, *J* = 6.1, 12.4 Hz, H-7′), 3.88 (6H, *s*, OMe-3′, 4′), 4.31 (1H, *d*, *J* = 9.5 Hz, H-7), 5.95 (2H, *s*, OCH_2O-3,4), 6.7-6.9 (6H, *m*, H-2, H-5, H-6, H-2′, H-5′, H-6′). ^{13}C NMR (50 MHz) (DEPT): δ 148.7 (C, C-3′), 147.7 (C, C-3), 147.0 (C, C-4′), 146.9 (C, C-4), 138.3 (C, C-1), 134.0 (C, C-1′), 120.9 (CH, C-6′), 120.3 (CH, C-6), 112.2 (CH, C-2′), 110.9 (CH, C-5′), 107.9 (CH, C-2), 106.9 (CH, C-5), 100.9 (CH_2, OCH_2O-3,4), 77.1 (CH, C-7), 55.9 (Me, OMe), 55.8 (Me, OMe), 42.8 (CH, C-8), 41.8 (CH_2, C-7′), 33.7 (CH, C-8′), 12.8 (Me, C-9′), 10.1 (Me, C-9). EIMS *m/z* (rel. int.): 358

$[M]^+$ (11), 340 $[M-H_2O]^+$ (16), 178 (25), 151 (100), 149 (4).

4-*Hydroxy*-5-*methoxy*-3′,4′-*methylenedioxy*-2,7′-*cyclolignan*-7-*one* (**5a**). Viscous oil. IR ν_{max} cm^{-1}: 3400, 2900, 2850, 1670, 1600, 1540, 1425, 1365. UV λ_{max} nm: 228 (log ε 4.342), 275 (log ε 4.021), 308 (log ε 3.851), $\lambda_{max}^{MeOH+MeONa}$ nm: 225 (log ε 4.247), 248 (log ε 4.013), 292 (log ε 3.886), 342 (log ε 4.017). ^{1}H NMR (90 MHz): δ 0.95 (3H, *d*, J = 7 Hz, Me-8′), 1.30 (3H, *d*, J = 7 Hz, Me-8), 2.1-2.6 (2H, *m*, H-8, H-8′), 3.63 (1H, *d*, J = 9.5 Hz, H-7′), 3.93 (3H, *s*, OMe-5), 5.50 (1H, *br s*, OH-4), 5.96 (2H, *s*, OCH_2O-3′, 4′), 6.28 (1H, *s*, H-3), 6.50–6.85 (3H, *m*, H-2′, H-5′, H-6′), 7.56 (1H, *s*, H-6). EIMS *m/z* (rel. int.): 340 $[M]^+$ (29), 284 (26), 178 (74), 164 (100), 149 (57), 135 (13), 121 (18).

5-*Methoxy*-3′,4′-*methylenedioxy*-7-*oxo*-2,7′-*cyclo lignan*-4-*yl acetate* (**5b**). Oil. ^{1}H NMR (90 MHz): δ 0.94 (3H, *d*, J = 6.5 Hz, Me-8′), 1.30 (3H, *d*, J = 6.5 Hz, Me-8), 2.24 (3H, *s*, -OAc-4), 2.0–2.5 (2H, *m*, H-8, H-8′), 3.68 (1H, *d*, J = 9.8 Hz, H-7′), 3.87 (3H, *s*, OMe-5), 5.97 (2H, *s*, OCH_2O-3′,4′), 6.46 (1H, *s*, H-3), 6.50–6.85 (3H, *m*, H-2′, H-5′, H-6′), 7.60 (1H, *s*, H-6).

4-*Hydroxy*-5-*methoxy*-3′,4′-*methylenedioxy*-7′,8′-seco-2,7′-*cyclolignan*-7′,8′-*dione* (**6**). Viscous oil. $[\alpha]_D$ +25° ($CHCl_3$; *c* 0.14). IR ν_{max} cm^{-1}: 3400, 2930, 1700, 1640, 1600, 1500, 1489, 1430, 1260, 745. UV λ_{max} nm:235 (log ε 4.436), 280 (log ε 4.011), 320 (log ε 4.268); $\lambda_{max}^{MeOH+MeONa}$ nm: 232 (log ε 4.398), 275 (log ε 4.220), 310 (log ε 4.123), 380 (log ε 3.545). ^{1}H NMR (200 MHz): δ 1.03 (3H, *d*, J = 6.3 Hz, H-9), 2.03 (3H, *s*, H-9′), 2.55 (1H, *dd*, J = 6.3, 12.5 Hz, H-7), 2.95 (1H, *sxt*, J = 6.3 Hz, H-8), 3.05 (1H, *dd*, J = 6.3, 12.5 Hz, H-7), 3.93 (3H, *s*, OMe-5), 5.6 (1H, *s*, OH-4), 6.06 (2H, *s*, OCH_2O-3′, 4′), 6.74 (1H, *s*, H-6), 6.81 (1H, *d*, J = 8.0 Hz, H-5′), 6.89 (1H, *s*, H-3), 7.30 (2H, *m*, H-2′, H-6′). ^{13}C NMR (50 MHz) (DEPT): δ 212.7 (C, C-8′), 195.9 (C, C-7′), 151.8 (C, C-5), 148.0 (C, C-3′), 147.7 (C, C-4′), 142.9 (C, C-4), 132.8 (C, C-2), 132.2 (C, C-1), 131.1 (C, C-1′), 127.1 (CH, C-6), 115.8 (CH, C-3), 113.5 (CH, C-6), 109.5 (CH, C-5′), 107.7 (CH, C-2′), 101.9 (CH_2, OCH_2O-3′, 4′), 56.0 (Me, OMe-5), 48.8 (CH, C-8), 36.1 (CH_2, C-7), 29.1 (Me, C-9′), 16.4 (Me, C-9). EIMS *m/z* (rel. int.): 356 $[M]^+$ (17), 313 [M-MeCO]$^+$ (28), 285 (31), 284 (100), 149 (39), 121 (10).

5-*Methoxy*-3′,4′-*methylenedioxy*-2,7′-*cyclolignan*-4,7′,8′-*triol* (**7**). Viscous oil. $[\alpha]_D$ −28.5° ($CHCl_3$; *c* 0.18). IR ν_{max}^{KBr} cm^{-1}: 3410, 2900, 1600, 1500, 1430, 1235, 1100, 1030, 805, 780. UV λ_{max} nm: 230 (log ε 3.932), 282 (log ε 3.801); $\lambda_{max}^{MeOH+MeONa}$ nm: 225 (log ε 3.936), 236 (sh, log ε 3.881), 286 (log ε 3.788). ^{1}H NMR (200 MHz): δ 0.82 (3H, *s*, H-9′), 1.15 (3H, *d*, J = 6.8 Hz, H-9), 2.5–3.0 (3H, *m*, 2H-7, H-8), 3.84 (3H, *s*, OMe-5), 5.40 (1H, *br s*, OH-4), 5.95 (2H, *s*, OCH_2O-3′, 4′), 6.55 (1H, *s*, H-3), 6.61 (1H, *s*, H-6), 6.7–6.9 (3H, *m*, H-2′, H-5′, H-6′). ^{13}C NMR (50 MHz) (DEPT): δ 146.5 (C, C-3′, C-4′), 143.9 (C, C-3, C-4), 136.6 (C, C-1′), 133.5 (C, C-2), 129.0 (C, C-1), 122.6 CH, C-6′), 116.2 (CH, C-3), 110.3 (CH, C-6), 109.9 (CH, C-5′), 106.7 (CH, C-2′), 100.9 (CH_2, OCH_2O-3′, 4′), 80.4 (C, C-7′), 75.9 (C, C-8′), 55.9 (Me, OMe-5), 36.7 (CH_2, C-7), 34.5 (CH, C-8), 17.3 (Me, C-9′), 15.0 (Me, C-9). EIMS *m/z* (rel. int.): 358 $[M]^+$ (13), 340 $[M-H_2O]^+$ (12), 322 $[M-2H_2O]^+$ (10), 298 (95), 297 (100), 286 [M − MeCHCOHMe]$^+$ (17), 285 (30), 149 (25), 121 (8).

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