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Studies on the Constituents of Ophiopogonis Tuber. VI.[1] Structures of Homoisoflavonoids. (2)

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New homoisoflavonoidal compounds, ophiopogonone A(Va), isoophiopogonone A(VIa), ophiopogonone *B*(VIIa), desmethylisoophiopogonone B(VIII) and ophiopogonanone A(IXa) were isolated as constitutents of Ophiopogonis tuber (*Ophiopogon japonicus* Ker-Gawler var. *genuinus* Maxim., Liliaceae), and their chemical structures were elucidated as 5,7-dihydroxy-6-methyl-3-(3,4-methylenedioxybenzyl)chromone, 5,7-dihydroxy-8-methyl-3-(3,4-methylenedioxybenzyl)chromone, 5,7-dihydroxy-6-methyl-3-(4-methoxybenzyl)chromone, 5,7-dihydroxy-8-methyl-3-(4-hydroxybenzyl)chromone and 5,7-dihydroxy-6-methyl-3-(3,4-methylenedioxybenzyl)chroman-4-one, respectively, by chemical and spectral studies.

Keywords——Ophiopogonis tuber; *Ophiopogon japonicus*; Liliaceae; homoisoflavonoids; ophiopogonones A, B; isoophiopogonone A; desmethylisoophiopogonone B; ophiopogonanone A; UV; PMR

In the preceding paper,[1] we reported the isolation of eleven new homoisoflavonoids from Ophiopogonis tuber (tuber of *Ophiopogon japonicus* Ker–Gawler var. *genuinus* Maxim., Liliaceae), and described the determination of the structures of methylophiopogonanones A (I) and B (II), and methylophiopogonones A (III) and B (IV) which have two methyl groups in the same aromatic ring. Among them, III and IV were the first examples of naturally occurring homoisoflavones having a double bond at C_{2-3}, while I and II were ordinary homoisoflavanones.

The present paper is mainly concerned with ophiopogonone A (Va), isoophiopogonone A (VIa), ophiopogonone B (VIIa), desmethylisoophiopogonone B (VIII) and ophiopogonanone A (IXa).

Ophiopogonone A (Va)

Va, $C_{18}H_{14}O_6$ (MW. 326), was obtained as pale yellow needles, and the molecular formula was supported by mass spectrometry. The infrared (IR) spectrum showed absorption bands at 3300 (OH), 1640 (C=O) and 937 (methylenedioxy) cm^{-1}, and the ultraviolet (UV) spectrum exhibited absorption maxima corresponding to a homoisoflavonoidal structure.[1] The proton magnetic resonance (PMR) spectrum revealed the presence of an aromatic methyl group at δ 2.06 (3H, singlet), a benzylmethylene at δ 3.65 (2H, s.), a methylenedioxy group at δ 5.92 (2H, s.), aromatic ABC type protons centered at δ 6.76 (3H, multiplet),[1] an olefinic proton at δ 7.93 (1H, s.) and hydroxyl proton at δ 13.04 (1H, s., exchangeable with D_2O). Methylation of Va with ethereal diazomethane afforded a monomethyl ether (Vb) whose PMR spectrum showed a methoxyl signal at δ 3.86 (3H, s.), while acetylation of Va with acetic anhydride and pyridine gave a diacetate (Vd) (OAc at δ 2.37 and 2.50). This result and a PMR signal at δ 13.04 (1H, s.) indicated the presence of a chelated hydroxyl group assignable to C-5.

1) Part V: A. Tada, R. Kasai, T. Saitoh, and J. Shoji, *Chem. Pharm. Bull.*, **28**, 1477 (1980).
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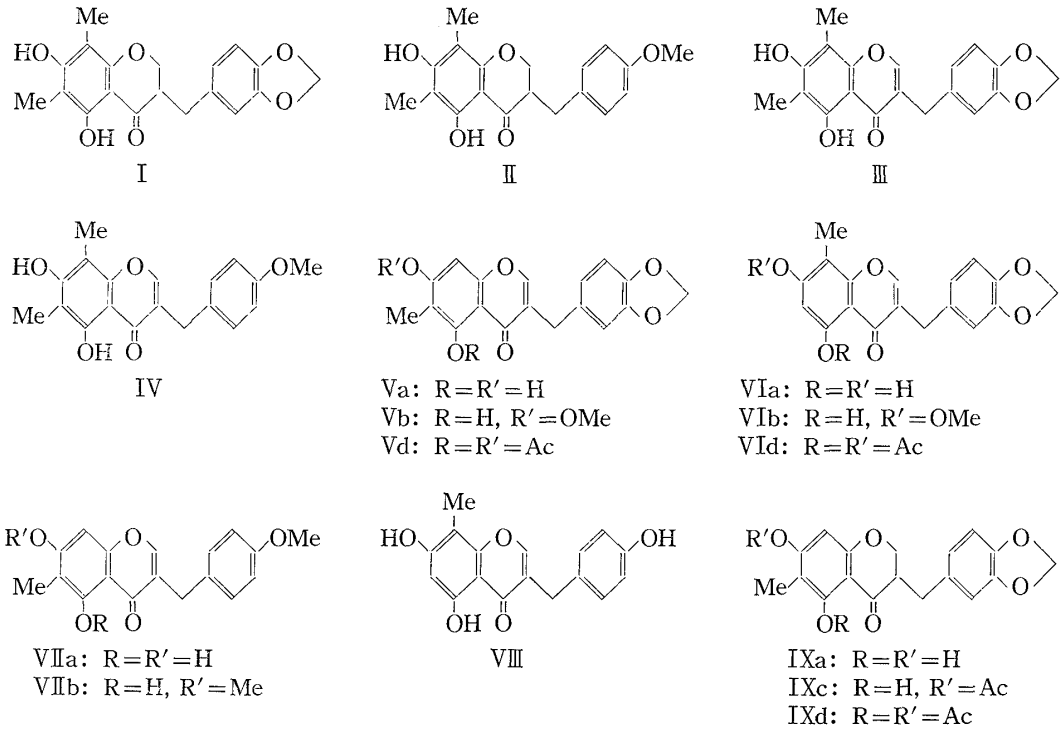

Chart 1

Besides these results, bathochromic shifts of the UV absorption upon addition of $AlCl_3$ and NaOAc supported the presence of hydroxyl groups at C-5 and C-7. Consequently, the location of the methylenedioxy group was inferred to be on the B-ring (side phenyl). The remaining signal at δ 6.42 (1H, s.) of Va (δ 7.16 in the case of Vd) may be assignable to either a C-8 or C-6 proton, and the former assignment seems reasonable on comparing this with the chemical shifts of C-8 protons in related compounds: kaempferol (δ 6.14 C_6–H; 6.42 C_8–H), licoflavonol (δ 6.45 C_8–H), kaempferol tetraacetate (δ 6.83 C_6–H; 7.26 C_8–H), licoflavonol tetraacetate (δ 7.25 C_8–H), and des-O-methylanhydroicarithin tetraacetate (δ 6.86 C_6–H).[3] Furthermore, Markham and Mabry[4] have pointed out that the chemical shift ranges of C_6- and C_8-isoflavonoidal aromatic C-methyl protons were δ 2.04—2.27 ppm and 2.14—2.45 ppm, respectively. Thus, the location of the methyl group giving a signal at δ 2.06 in the PMR spectrum was concluded to be at C-6.

Based on these results, the structure of ophiopogonone A is concluded to be Va, 5,7-dihydroxy-6-methyl-3-(3,4-methylenedioxybenzyl)chromone.

Isoophiopogonone A (VIa)

VIa, $C_{18}H_{14}O_6$ (MW. 326), was obtained as yellow needles, and the molecular formula was supported by mass spectrometry. The IR spectrum showed absorption bands at 3300 (OH), 1648 (C=O) and 928 (methylenedioxy) cm^{-1} and the UV spectrum exhibited absorption maxima corresponding to a homoisoflavonoidal structure. The PMR spectrum revealed the presence of an aromatic methyl group at δ 2.15 (3H, s.), a benzylmethylene at δ 3.71 (2H, s.), a methylenedioxy group at δ 5.97 (2H, s.), aromatic ABC type protons centered at δ 6.83 (3H, m.), an olefinic proton at δ 8.13 (1H, s.) and a hydroxyl proton at δ 12.79 (1H, s., exchangeable with D_2O). Although methylation with ethereal diazomethane afforded a monomethyl ether (VIb)

3) T. Saitoh, T. Kinoshita, and S. Shibata, *Chem. Pharm. Bull.*, **24**, 1242 (1976).
4) K.R. Markham and T.J. Mabry, "The Flavonoids," ed. by J.B. Harborne, T.J. Mabry, and H. Mabry, Chapman and Hall Ltd., London, 1975, p. 62.

Table I. PMR Spectral Data for Various Homoisoflavonoidal Compounds (δ Value, 90 MHz)

Compound	C(2)	C(3)	C(5) OH	C(5) $OCOCH_3$	C(6) H	C(6) CH_3	C(7) OCH_3	C(7) $OCOCH_3$	C(8) H	C(8) CH_3	C(9)	C(2′, 5′, 6′)	C(2′, 6′)	C(3′, 5′)	C(4′) OCH_3	C(7′)
Va[a]	7.93		13.04			2.06			6.42		3.65	6.66—6.86 m				5.92
Vb	7.43		12.68			2.08	3.86		6.30		3.67	6.71 br.				5.90
Vd	7.43			2.50		2.09		2.37	7.16		3.70	6.67—6.86 m				5.97
VIa[a]	8.13		12.79		6.37					2.15	3.71	6.72—6.93 m				5.97
VIb	7.57		12.67		6.43		3.91			2.12	3.71	6.78 br.				5.96
VId	7.43			2.41	6.77			2.32		2.18	3.71	6.61—6.79 m				5.91
VIIa[a]	8.00		13.19			2.08			6.49		3.71		7.34 d (J=9 Hz)	6.90 d (J=9 Hz)	3.81	
VIIb	7.41		12.65			2.08	3.76 or 3.85		6.29		3.68		7.04 d (J=9 Hz)	6.80 d (J=9 Hz)	3.76 or 3.85	
VIII[a]	8.00		12.74		6.36					2.16	3.68		7.20 d (J=9 Hz)	6.80 d (J=9 Hz)		
IXa[a]	4.08 d.d (J_1=12 Hz J_2=7 Hz) 4.32 d.d (J_1=12 Hz J_2=3 Hz)	2.78—3.07 m	12.40			1.98			6.00		2.68 d.d (J_1=12 Hz J_2=9.5 Hz) 3.13 d.d (J_1=12 Hz J_2=3 Hz)	6.73—6.88 m				5.96
IXc	4.11 d.d (J_1=12 Hz J_2=7 Hz) 4.32 d.d (J_1=12 Hz J_2=3 Hz)	2.78—3.02 m	12.20			1.99		2.32	6.19		2.69 d.d (J_1=12 Hz J_2=9.5 Hz) 3.08 d.d (J_1=12 Hz J_2=3 Hz)	6.71 br.				5.93
IXd	4.11 d.d (J_1=12 Hz J_2=7 Hz) 4.32 d.d (J_1=12 Hz J_2=3 Hz)	2.67—3.03 m		2.42		1.93		2.32	6.70		2.68 d.d (J_1=12 Hz J_2=9.5 Hz) 3.14 d.d (J_1=12 Hz J_2=3 Hz)	6.53—6.78 m				5.92

a) These compounds were measured in acetone-d_6 and others were measured in $CDCl_3$.
Hydroxyl signals of all compounds were confirmed by the addition of D_2O.
d=doublet, m=multiplet; others not specified are singlets.

whose PMR spectrum showed a 3H singlet at δ 3.91, acetylation of VIa with acetic anhydride and pyridine gave a diacetate (VId) (OAc at δ 2.32 and 2.41). The UV spectrum of VIa indicated the presence of 5- and 7-hydroxy groups, since marked bathochromic shifts were observed upon addition of $AlCl_3$ and NaOAc. Based on these data, VIa was characterized as a homoisoflavone having the same functional groups as Va, namely a positional isomer of Va. Based on the chemical shifts of the C-6 and C-8 protons and those of isoflavonoidal aromatic C-methyl protons of related compounds, the remaining signal of VIa at δ 6.37 (1H, s.) (δ 6.77 of VId) and δ 2.15 (3H, s.) (δ 2.18 of VId) were assigned to an aromatic proton at C-6 and aromatic C-methyl protons at C-8, respectively. Thus, the structure of isoophiopogonone A was concluded to be VIa 5,7-dihydroxy-8-methyl-3-(3,4-methylenedioxybenzyl)-chromone.

Ophiopogonone B (VIIa)

Ophiopogonones A (Va) and B (VIIa) were obtained as a mixture showing a homogeneous spot on a TLC plate, and VIIa was isolated by column chromatography on Sephadex LH-20, eluting with ethanol.

VIIa, $C_{18}H_{16}O_5$ (MW. 312), was crystallized as pale yellow needles. The IR spectrum showed absorption bands at 3300 (OH), 1645 and 1626 (C=O) cm^{-1}, and the UV spectrum exhibited absorption maxima corresponding to a homoisoflavonoidal structure. The PMR spectrum revealed the presence of an aromatic C-methyl group at δ 2.08 (3H, s.), a benzylmethylene at δ 3.71 (2H, s.), a methoxyl group at δ 3.81 (3H, s.), an aromatic proton at δ 6.49 (1H, s.), four aromatic protons of an A_2B_2 system at δ 6.90 and 7.34 (2H each, d., J=9 Hz), an olefinic proton at δ 8.00 (1H, s.), and a hydroxyl proton at δ 13.19 (1H, s., exchangeable with D_2O). Treatment of VIIa with ethereal diazomethane afforded a monomethyl ether (VIIb) whose PMR spectrum showed methoxyl groups at δ 3.76 and 3.85 (3H each ,s.) and a hydroxyl group at δ 12.65 (1H, s., exchangeable with D_2O). The UV spectrum of VIIa indicated the presence of 5- and 7-hydroxyl groups, since remarkable bathochromic shifts were observed upon addition of $AlCl_3$ and NaOAc. The structure VIIa of ophiopogonone B was finally deduced by comparison of the chemical shifts of aromatic C-methyl protons and the aromatic singlet proton with those of Va and VIa. Consequently, ophiopogonone B was concluded to be VIIa, 5,7-dihydroxy-6-methyl-3-(4-methoxybenzyl)chromone.

Desmethylisoophiopogonone B (VIII)

VIII, $C_{17}H_{14}O_5$ (MW. 298), was obtained as a pale yellow powder. The IR spectrum showed absorption bands at 3400 (OH) and 1650 (C=O) cm^{-1}, and the UV spectrum exhibited absorption maxima corresponding to a homoisoflavonoidal structure. The PMR spectrum revealed the presence of an aromatic methyl group at δ 2.16 (3H, s.), a benzylmethylene at δ 3.68 (2H, s.), an aromatic proton at δ 6.36 (1H, s.), four aromatic protons of an A_2B_2 system at δ 6.80 and 7.20 (2H each, d., J=9 Hz), an olefinic proton at δ 8.00 (1H, s.) and a hydroxyl proton at δ 12.74 (1H, s., exchangeable with D_2O). The UV absorption shifts upon addition of $AlCl_3$ and NaOAc indicated the presence of two hydroxyl groups at C-5 and C-7. The structure of desmethylisoophiopogonone B was deduced by comparison of its chemical shifts of aromatic C-methyl protons and an aromatic proton with those of Va, VIa and VIIa. The structure of the B-ring (side phenyl) was inferred by analysis of the mass spectrum show in Chart 2. Based on these results, the structure of desmethylisoophiopogonone B was concluded to be VIII, 5,7-dihydroxy-8-methyl-3-(4-hydroxylbenzyl)chromone.

Ophiopogonanone A (IXa)

IXa, $C_{18}H_{16}O_6$ (MW. 328), $[\alpha]_D$ $-13.0°$ (dioxane), was obtained as colorless needles. The IR spectrum showed absorption bands at 3400 (OH), 1635 (C=O) and 928 (methylenedioxy) cm^{-1}, and the UV spectrum exhibited absorption maxima corresponding to a homoisoflavanoidal structure.[1] The PMR spectrum revealed the presence of an aromatic methyl

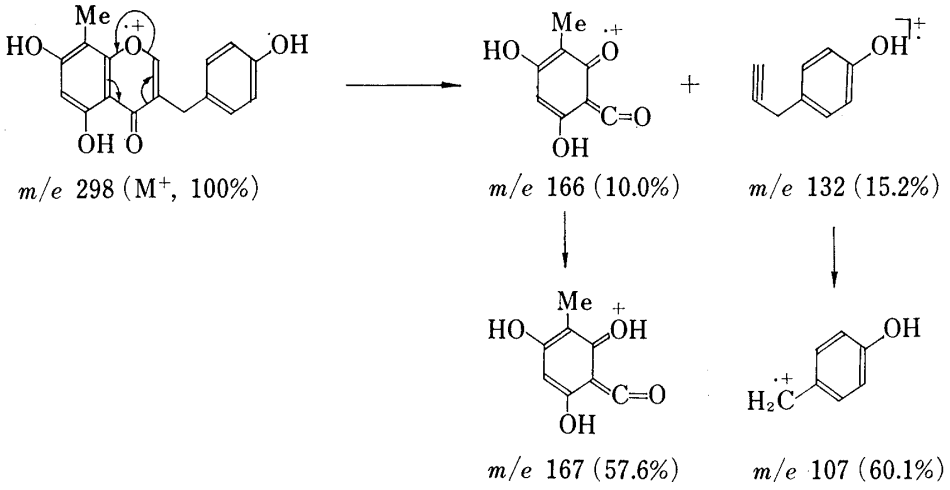

Chart 2. Mass Spectrum of Desmethylisoophiopogonone B (VIII)

group at δ 1.98 (3H, s.), a methylenedioxy group at δ 5.96 (2H, s.), an aromatic proton at δ 6.00 (1H, s.) and ABC-type aromatic proton signals centered at δ 6.81 (3H, m.), a hydroxyl proton at δ 12.40 (1H, s., exchangeable with D_2O), a pair of benzylmethylene protons at δ 2.68 (1H, d. d., J_1=12 Hz, J_2=9.5 Hz) and 3.13 (1H, d. d., J_1=12 Hz, J_2=3 Hz), and γ-dihydropyrone protons at δ 2.78—3.07 (1H, m., H_{3ax}), 4.08 (1H, d. d., J_1=12 Hz, J_2=7 Hz, H_{2ax}) and 4.32 (1H, d. d., J_1=12 Hz, J_2=3 Hz, H_{2eq}). Besides these results, the occurrence of UV absorption shifts upon addition of $AlCl_3$ and NaOAc indicated the presence of two hydroxyl groups at C-5 and C-7. The usual acetylation gave a mixture of a monoacetate (IXc), whose PMR spectrum showed a 3H singlet at δ 2.32, and a diacetate (IXd) (OAc at δ 2.32 and 2.42). Ophiopogonanone A was suggested to be a homoisoflavanoidal compound, and the positions of the aromatic C-methyl group and an aromatic proton were deduced by comparison of the chemical shifts of IXc and IXd with those of Vd, VId and methyl-ophiopogonanone A diacetate.

Consequently, the structure of ophiopogonanone A was concluded to be IXa, 5,7-dihydroxy-6-methyl-3-(3,4-methylenedioxybenzyl)chroman-4-one.

Confirmation of the structures of these compounds has been provided by total synthesis, which will be reported in a forthcoming paper.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (hot-stage type) and are uncorrected. The UV spectra were recorded with a Hitachi EPS-3 spectrophotometer, IR spectra with a JASCO IRA-1 unit, and PMR spectra with a Hitachi R-22 (90 MHz) spectrometer. Mass spectra were measured with a Hitachi RMS-4 or a JEOL JMS-01SG high-resolution spectrometer with a direct inlet system. The optical rotations were measured with a Yanagimoto OR-50 polarimeter.

Ophiopogonone A(Va)——Pale yellow needles (from EtOH), mp 235—236°. UV λ_{max}^{EtOH} nm (log ε): 225 (4.21), 264 (4.24), 295 (4.05), 330 (inflection, 3.57); $\lambda_{max}^{EtOH+NaOAc}$ nm: 332. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 272, 316, 365. MS *m/e*: 326 (M^+, 100%), 167 (54.4%), 166 (4.1%), 160 (26.7%), 135 (9.3%). *Anal.* Calcd for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32. Found: C, 66.23; H, 4.32.

Isoophiopogonone A(VIa)——Yellow needles (from EtOH), mp 202—203°. UV λ_{max}^{EtOH} nm (log ε): 225 (4.32), 263 (4.52), 291 (4.02), 330 (3.69). $\lambda_{max}^{EtOH+NaOAc}$ nm: 265, 277, 333. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 273, 315, 368. MS *m/e*: 326 (M^+, 100%), 167 (28.2%), 160 (14.5%), 135 (4.3%). *Anal.* Calcd for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32. Found: C, 65.96; H, 4.36.

Ophiopogonone B(VIIa)——Pale yellow needles (from EtOH), mp 235—237°, UV λ_{max}^{EtOH} nm (log ε): 226 (4.30), 262 (4.25), 298 (3.93), 330 (inf. 3.62). $\lambda_{max}^{EtOH+NaOAc}$ nm: 332. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 271, 318, 370. MS *m/e*: 312 (M^+, 100%), 167 (42.2%), 146 (22.2%), 121 (7.4%). *Anal.* Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 68.96; H, 5.16.

Desmethylisoophiopogonone B(VIII)——A pale yellow powder (from EtOH), mp 208—210°. UV λ_{max}^{EtOH} nm (log ε): 222 (4.24), 264 (4.29), 297 (3.94), 325 (inf. 3.94). $\lambda_{max}^{EtOH+NaOAc}$ nm: 332. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 316, 364. MS *m/e*: Calcd for $C_{17}H_{14}O_5$ 298.0841 (M^+). Found: 298.0842.

Ophiopogonanone A(IXa)——Colorless needles (from EtOH), mp 175—176°. $[\alpha]_D^{17}$ −13.0° (c=1.0, dioxane). UV λ_{max}^{EtOH} nm (log ε): 213 (4.14), 295 (4.28). $\lambda_{max}^{EtOH+NaOAc}$ nm: 335. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 318. MS m/e: 328 (M^+, 100%), 193 (4.9%), 167 (37.7%), 166 (16.4%), 162 (8.9%), 135 (100%). *Anal.* Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.78; H, 4.95.

Methylations of Va, VIa and VIIa with CH_2N_2——An excess of ethereal diazomethane was added to a methanolic solution of Va, VIa or VIIa at 0°, and the mixture was allowed to stand for 1 hr. The solvent was removed under reduced pressure and the residue was crystallized from EtOH.

Ophiopogonone A Monomethyl Ether(Vb): Colorless needles, mp 179—180°. UV λ_{max}^{EtOH} nm (log ε): 234 (4.10), 256 (4.07), 263 (4.09), 292 (3.84), 330 (inf. 3.39). $\lambda_{max}^{EtOH+AlCl_3}$ nm: 273, 315, 374. IR ν_{max}^{KBr} cm^{-1}: 3430, 1644, 1617, 926. MS m/e: 340 (M^+).

Isoophiopogonone A Monomethyl Ether(VIb): Colorless needles, mp 164—165°. UV λ_{max}^{EtOH} nm (log ε): 229 (4.16), 248 (4.23), 256 (4.32), 263 (4.35), 290 (3.87), 331 (3.59). UV $\lambda_{max}^{EtOH+AlCl_3}$ nm: 273, 315, 370. IR ν_{max}^{KBr} cm^{-1}: 3450, 1650, 1605, 930. MS m/e: 340 (M^+).

Ophiopogonone B Monomethyl Ether(VIIb): Colorless needles, mp 154—155°. UV λ_{max}^{EtOH} nm (log ε): 230 (4.00), 262 (4.01), 290 (3.82), 323 (inf. 2.82). $\lambda_{max}^{EtOH+AlCl_3}$ nm: 273, 315, 364. IR ν_{max}^{KBr} cm^{-1}: 3420, 1640, 1620, 1603. MS m/e: 326 (M^+).

Acetylations of Va, VIa and IXa with Ac_2O and Pyridine——Va, VIa and IXa were acetylated with acetic anhydride and pyridine in the usual manner. The prodxcts of IXa were purified by column chromatography on silicic acid, eluting with $CHCl_3$, to afford IXc and IXd. Each product was crystallized from EtOH.

Ophiopogonone A Diacetate(Vd): Colorless needles, mp 162—163°. UV λ_{max}^{EtOH} nm (log ε): 228 (4.82), 252 (sh. 4.55), 291 (4.25), 340 (3.93). IR ν_{max}^{KBr} cm^{-1}: 1757, 1642, 1614, 925. MS m/e: 410 (M^+).

Isoophiopogonone A Diacetate(VId): Colorless needles, mp 133—134°. UV λ_{max}^{EtOH} nm (log ε): 227 (4.24), 295 (3.68), 308 (inf. 3.57). IR ν_{max}^{KBr} cm^{-1}: 1772, 1641, 1624, 1610, 928. MS m/e: 410 (M^+).

Ophiopogonanone A Monoacetate(IXc): Colorless needles, mp 151—152°. $[\alpha]_D^{24}$ −27.4° (c=0.1, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 225 (4.18), 282 (4.04). $\lambda_{max}^{EtOH+AlCl_3}$ nm: 238, 308. IR ν_{max}^{KBr} cm^{-1}: 3450, 1752, 1640, 928. MS m/e: 370 (M^+).

Ophiopogonanone A Diacetate(IXd): Colorless needles, mp 110—111°. $[\alpha]_D^{24}$ −5.9° (c=0.5, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 218 (4.48), 260 (4.04), 288 (3.83). IR ν_{max}^{KBr} cm^{-1}: 1760, 1685, 1618, 925. MS m/e: 412 (M^+).

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