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The Constituents of *Scirpus fluviatilis*(Torr.) A. Gray. I. The Structures of Two New Hydroxystilbene Dimers, Scirpusin A and B

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Two new hydroxystilbene dimers, named scirpusin A(**1**) and B(**12**), were isolated from the rhizoma of *Scirpus fluviatilis* (Torr.) A. Gray (Cyperaceae) together with two known hydroxystilbenes, resveratrol and 3,3′,4,5′-tetrahydroxystilbene, and four known triterpens, betulin, lupeol, betulinaldehyde and betulinic acid. The structures of scirpusin A and B were elucidated to be **1** and **12**, respectively, by chemical and spectral evidence.

Keywords——*Scirpus fluviatilis* (Torr.) A. Gray; Cyperaceae; hydroxystilbene dimer; scirpusin A; scirpusin B; ^{13}C NMR

Scirpus fluviatilis (Torr.) A. Gray is a perennial plant growing in ponds and swamps, and its rhizoma are used as the emmenagogue, galactagogue and antispasmodic under the

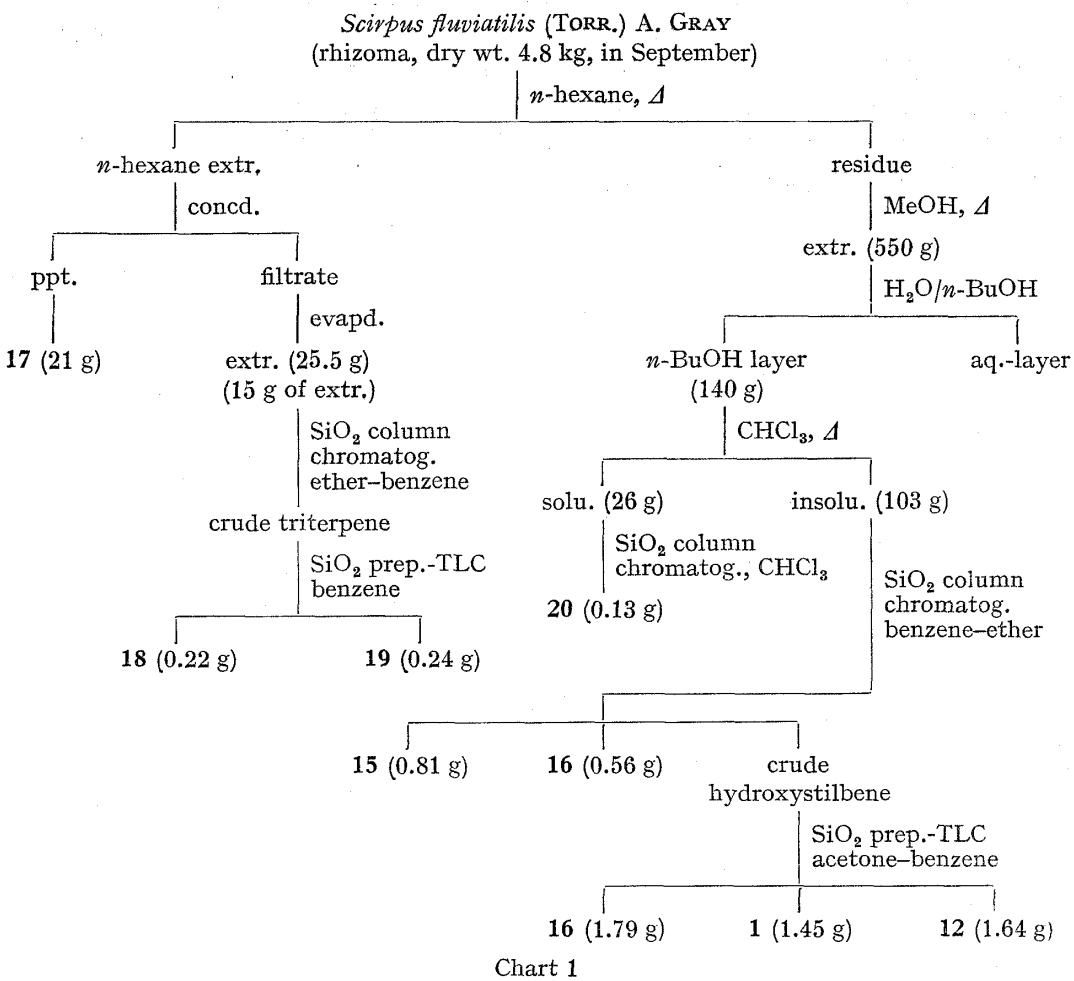

Chart 1

1) Location: *1421, Izumi, Komae-shi, Tokyo 182, Japan.*

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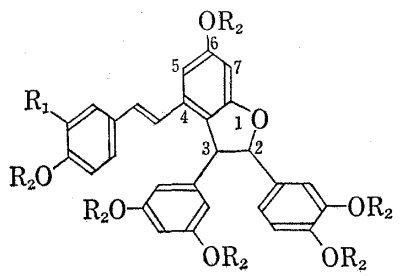
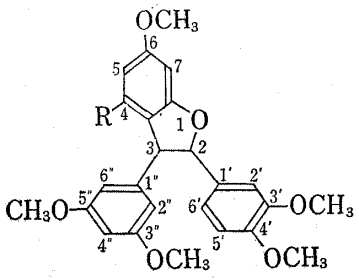
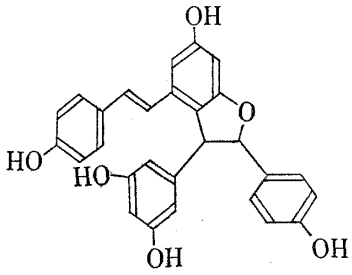
names of Kei-san-ryo (荊三稜) in Japan and Ching-san-leng in China.[2] This paper concerns the structures of two new hydroxystilbene dimers, named scirpusin A(**1**) and B(**12**), isolated from the rhizoma of this plant together with four known triterpens, betulin (**17**), lupeol (**18**), betulinaldehyde (**19**)[3] and betulinic acid (**20**), and two known stilbene derivatives, resveratrol (**15**) and 3,3′,4,5′-tetrahydroxystilbene (**16**).



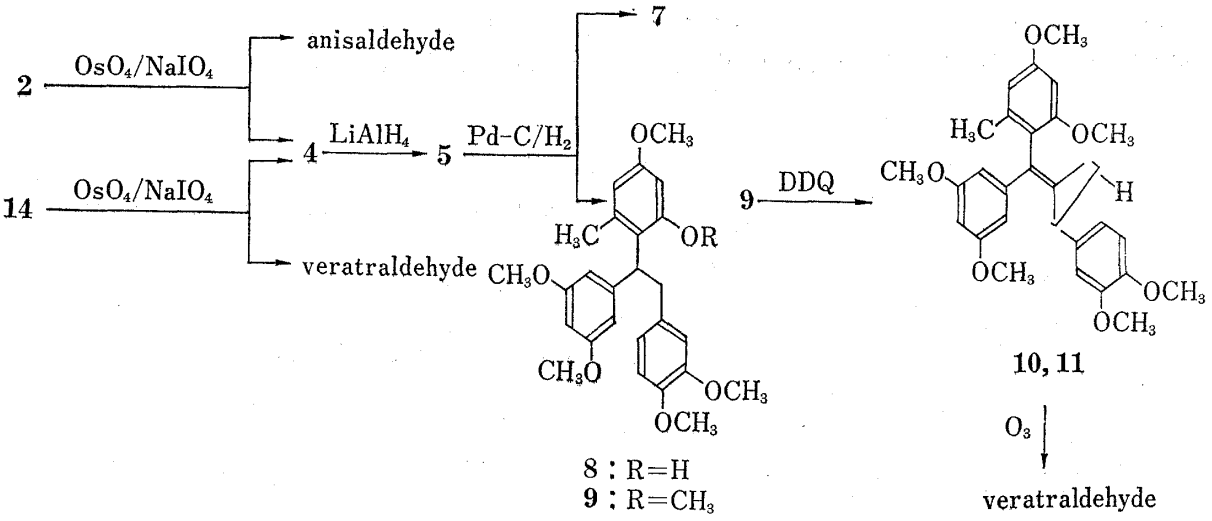
1 : R_1=H, R_2=H
2 : R_1=H, R_2=CH_3
3 : R_1=H, R_2=$COCH_3$
12 : R_1=OH, R_2=H
13 : R_1=$OCOCH_3$, R_2=$COCH_3$
14 : R_1=OCH_3, R_2=CH_3



4 : R=CHO
5 : R=CH_2OH
6 : R=CH_2OOC–C$_6$H$_4$–Br
7 : R=CH_3

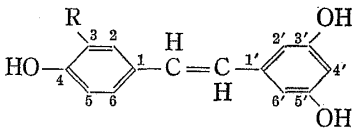


ε-viniferin

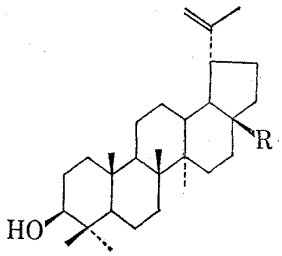


8 : R=H
9 : R=CH_3

Chart 2



15: R=H
16: R=OH



17: R=CH_2OH
18: R=CH_3
19: R=CHO
20: R=COOH

Chart 3

2) G.A. Stuart, "Chinese Materia Medica," printed at The American Presbyterian Mission Press, Shianghai. 1911, pp. 141—142; K. Shibata (ed.), "Cyclopedia of Useful Plants and Plant Products" the 5th ed., Hokuryukan, Tokyo, 1957, pp. 62—63; E. Koizumi, "Wakanyakkō," the 7th ed., Nankōdō, Tokyo, 1941, Vol. II, pp. 248—250; K. Akamatsu, "Wakanyaku," Ishiyakushuppan, Tokyo, 1970, p. 612.
3) H. Rimpler, H. Kuhn, and Ch. Leuckert, *Arch. Pharm.*, **299**, 422 (1966); M. Yasue, Y. Kato, M. Sugimoto, and J. Sakakibara, *Yakugaku Zasshi*, **89**, 1736 (1969).

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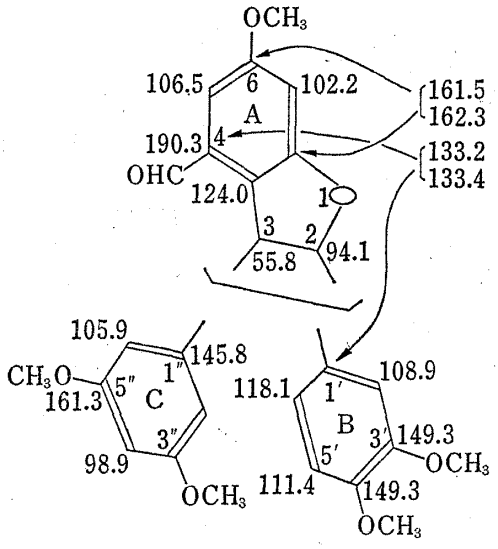
The procedure for the isolation of the constituents of the plant including scirpusin A and B is shown in Chart 1 and in the experimental section.

Scirpusin A (**1**, yield 0.03%), $C_{28}H_{22}O_7$, a pale brown amorphous powder, gives a dark green colouration with ferric chloride in ethanol and emits a blue fluorescence under an ultraviolet (UV) lamp (365 nm). The UV spectrum with the absorption maxima at 224 (log ε: sh 4.56), 290 (sh 4.27), 311 (sh 4.36) and 323 nm (4.39) and the infrared (IR) spectrum (in KBr) with the band at 960 cm^{-1} suggested that **1** should contain a *trans*-stilbene moiety.[4a,5] The proton nuclear magnetic resonance (^1H NMR) spectrum of **1** (in d_6-acetone) reveals an A_2B_2 type quartet at δ 6.73 and 7.20 (J=9 Hz) indicating the presence of a para substituted aromatic ring and an AB type quartet at δ 4.46 and 5.40 (J=6 Hz). The D_2O exchangeable broad signal at δ 8.16 (6×H) indicates that **1** is a polyphenolic compound. Treatment of **1** with ethereal diazomethane and then with methyl iodide in the presence of silver oxide in dimethylformamide yielded a hexamethyl ether (**2**) as an amorphous powder, $C_{34}H_{34}O_7$ (M^+, m/e, 554), and acetylation of **1** with acetic anhydride in pyridine yielded a hexaacetate (**3**), $C_{40}H_{34}O_{13}$, colourless needles, mp 174.5—176.5°. The ^1H NMR spectrum of **2** (in D_6-acetone) shows six methoxyls around δ 3.75—3.92 and the complicated signals around δ 6.7—7.4 assignable to the aromatic and olefinic protons (total: 14×H). The appearance of an AB type quartet at δ 4.65 and 5.54 (J=6 Hz) suggests the presence of the 2,3-diaryldihydrobenzofuran nucleus in **2** comparing with ε-viniferin, which is a first hydroxystilbene dimer isolated from the grape vine leaves infected with *Botrytis cinerea* or irradiated with a UV light.[4a,b] The ^1H NMR spectrum of **3** reveals six acetoxyls around δ 2.00—2.26 and an AB type quartet at δ 4.93 and 5.72 (J=6 Hz). On the basis of the above data, it was suggested that **1** should be a stilbene dimer having six phenolic hydroxyls and 2,3-diaryldihydrobenzofuran nucleus.



OCH_3=55.3(2C) and 56.1(3C)

4'

Chart 4. ^{13}C NMR Spectrum was taken in $CDCl_3$ at 25° (δ value)

Oxidation of **2** by the Lemieux-Johnson's method ($OsO_4/NaIO_4$ in dioxane-H_2O)[6] afforded anisaldehyde and an aldehyde **4**, $C_{26}H_{26}O_7$, whose ^1H NMR spectrum (in $CDCl_3$) shows five methoxyls, an AB type quartet (δ 4.98 and 5.63, J=6 Hz), eight aromatic protons [δ 6.42 (3H, s) and 6.8—7.1 (5H, m)] and an aldehyde proton (δ 9.90, s). The carbon (^{13}C) NMR spectral analysis of **4** (in $CDCl_3$) comparing with those of neolignans,[7] flavonoids,[8] ε-viniferin[4a,b] and the others,[9] indicated that **4** has the partial structure **4'** (Chart 4). Two protonated aromatic carbons at δ 98.9 and 102.2, which are much more shielded than the other protonated aromatic carbons, can be assigned to C-4'' and C-7 carbons. The protonated aromatic carbons at δ 105.9 (2×C) and non-

4) *a*) P. Langcake and R.J. Pryce, *Experintia*, **33**, 151 (1977); *b*) *Idem*, *Chem. Commun.*, **1977**, 208; *c*) R.J. Pryce and P. Langcake, *Phytochemistry*, **16**, 1452 (1977).
5) M. Klaar and W. Steglich, *Chem. Ber.*, **110**, 1063 (1977).
6) R. Pappo, P.S. Allen, R.U. Lemieux, and W.S. Johnson, *J. Org. Chem.*, **21**, 478 (1956).
7) C.J. Kelley, R.C. Harruff, and M. Carmark, *J. Org. Chem.*, **41**, 449 (1976); E. Wenkert, H.E. Gottlieb, O.R. Gottlieb, M.O.Da.S. Pereira, and M.D. Formiga, *Phytochemistry*, **15**, 1547 (1976).
8) C.A. Kingsbury and J.H. Looker, *J. Org. Chem.*, **40**, 1120 (1975).
9) A. Arnone, L. Camarda, L. Merlini, and G. Nasini, *J. Chem. Soc. Perkin I*, **1977**, 2118; H. Wagner, V.M. Chari, M. Seitz, and I. Riess-Maurer, *Tetrahedron Lett.*, **1978**, 381.

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protonated carbons at 161.3 (2×C), which are assignable to C-2″,6″ and C-3″,5″, respectively, indicates the presence of the ring C. Three doublet signals at δ 55.8, 94.1 and 190.3 are assigned to C-3, C-2 and an aldehyde carbon, respectively. Furthermore, the aldehyde doublet ($^1J_{C-H}$=176.7 Hz) in the undecoupled spectrum shows an additional three bond coupling ($^3J_{C-H}$=*ca.* 4.5 Hz) to provide a double doublet as the result of a coupling interaction with one *ortho*-proton. The other signals shows good agreement with the partial structure **4′**.

Next, reduction of **4** with lithium aluminium hydride ($LiAlH_4$) in dry ether afforded an alcohol **5**, $C_{26}H_{28}O_7$, colourless needles, mp 124.5—127°, which gave a *p*-bromobenzoate (**6**), $C_{33}H_{31}BrO_9$, colourless needles, mp 104.5—106°, by treatment with *p*-bromobenzoyl chloride in the presence of potassium carbonate in acetone. On catalytic hydrogenation over 10% palladized charcoal in methanol containing 3% acetic acid, **5** afforded compound **7**, $C_{26}H_{28}O_6$, colourless needles, mp 101.5—102.5°, and compound **8**, $C_{26}H_{30}O_6$, colourless prisms, mp 111.5—113.5°. Compound **7** shows the signals of an aromatic methyl (δ 1.86, s), $C_{(3)}$ and $C_{(2)}$-protons (δ 4.33 and 5.42, each d, J=6 Hz), and five methoxyls in the ^{1}H NMR spectrum (in $CDCl_3$) and shows no hydroxyl band in the IR spectrum. Compound **8** reveals a strong hydroxyl band (3430 cm^{-1}) in the IR spectrum and gives a positive Gibbs test (deep blue). The ^{1}H NMR spectrum of **8** shows an aromatic methyl (δ 1.83, s), a benzylic methylene (δ 3.20—3.60, m) and a methine (δ 4.43, m) signals, and it lacks the AB type quartet observed in that of **5**, indicating the cleavage of the dihydrofuran ring.

Methylation of **8** with dimethyl sulfate followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dry benzene[10] afforded two olefinic compounds, **10**, $C_{27}H_{30}O_6$, mp 127—129°, and **11**, $C_{27}H_{30}O_6$, amorphous. The ^{1}H NMR spectra of these compounds show the complicated nine proton signals around δ 6.2—7.0 and no methylene and methine signals. Especially, in **11**, one proton is observed at the lower field (δ 7.10) than the other eight aromatic protons, indicating the presence of the trisubstituted ethylene moiety. Although the structures of **10** and **11** could not be clarified, they are assumed to be the geometrical isomers represented as the structures in Chart 2. Finally, ozonolysis of **10** and **11** (as a mixture) afforded an aldehyde, which was identified with veratraldehyde by the direct comparison [mixed mp, IR (as 2,4-DNP-derivative) and ^{1}H NMR (as aldehyde)]. Thus, the structure of scirpusin A was elucidated to be **1**.

Scirpusin B (**12**, yield 0.034%), $C_{28}H_{22}O_8$, gives a dark green colouration with ferric chloride in ethanol and shows the absorption maxima at 225 (log ε: sh 4.55), 290 (4.17), 308 (sh 4.21) and 331 nm (4.32) in the UV spectrum. The heptaacetate (**13**) of **12**, $C_{42}H_{36}O_{15}$, colourless needles, mp 97—102°, reveals seven acetoxyls (δ 2.20—2.27), an AB type quartet at δ 4.93 and 5.70 (each 1H, J=6 Hz) assignable to $C_{(3)}$ and $C_{(2)}$ protons of the dihydrobenzofuran nucleus and the complicated signals of the aromatic and olefinic protons (total: 13×H) in the ^{1}H NMR spectrum (in D_6-acetone). Methylation of **12** by the same manner as in the case of **1** afforded a heptamethyl ether (**14**), $C_{35}H_{36}O_8$,[11] which was oxidized by the Lemieux-Johnson's method to give veratraldehyde and **4**. Thus, the structure of scirpusin B was elucidated to be **12**.

The configurations at the C-2 and C-3 positions in **1** and **12** could not be clarified from the coupling constants of $C_{(2)}$ and $C_{(3)}$ protons, since the ^{1}H NMR spectral characteristics of *cis*- and *trans*-2,3-substituted dihydrobenzofuran derivative are not at all certain as shown in the neolignans.[12] However, it was assumed that **1** and **12** were isolated as *dl*-forms on the basis of the circular dichroism (CD) spectra of **2** and **13**, which showed no absorption between 400—250 nm.

10) J.W.A. Findlay and A.B. Turner, "Organic synthesis," Coll. Vol. V, ed., H.E. Baumgarten, pp. 428—431, John Wiley and Sons, New York, 1973.
11) The presence of a *trans*-stilbene moiety in **14** was confirmed by the double resonance experiment in the ^{1}H NMR spectrum (see experimental section).
12) M. Gregson, W.D. Ollis, B.T. Redman, and I.O. Sutherland, *Chem. Commun.*, **1968**, 1394.

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Scirpusin A (**1**) and B (**12**) should be biosynthesized by oxidative dimerizations of **15** and **16** as postulated previously in biogenesis of the neolignans[13] and the styrylpyron dimer[5] (Chart 5). However, the possibility that **1** and **12** would be produced by UV light induced dimerization during the isolation process is unlikely, since the fresh methanolic extract prepared in a dark room revealed the presence of two new compounds (**1** and **12**) on the thin layer chromatogram.

Further investigation on the constituents of this plant including the possibility of the isolation of the hydroxystilbene oligomer such as α-viniferin[4a,c] and hopeaphenol[14] is under progress.

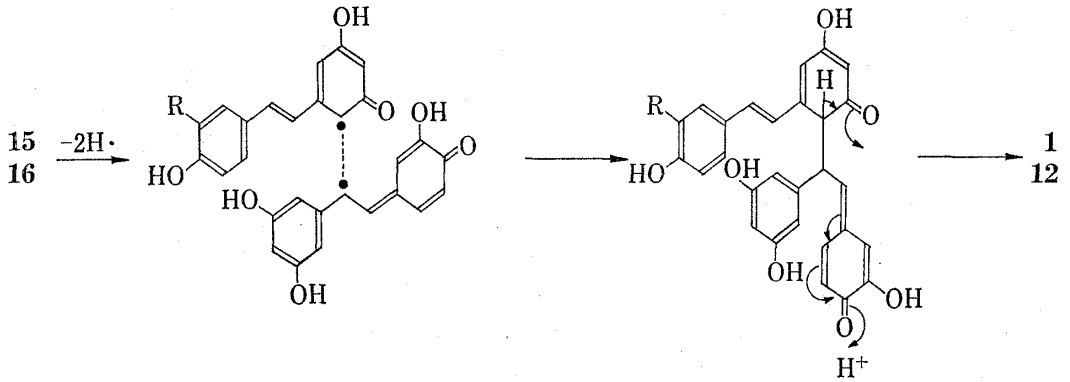

Chart 5. Possible Biogenetic Route to **1** and **12**

Experimental

All melting points were determined on a Yanagimoto Micromelting Point Apparatus (a hot stage type) and uncorrected. The UV spectra were recorded with a Hitachi Digital Spectrophotometer Model 624 and the IR spectra with a Hitachi Model EPI-G2. The ^{1}H NMR spectra were recorded with a Varian Model T-60 and JEOL Model PS-100, and ^{13}C NMR spectrum with a Varian Model FT-80 spectrometers. The Mass spectra were measured with a Hitachi Double Focussing Mass Spectrometer. The Specific Rotations were measured with a JASCO Model DIP-SL (Digital) and the CD spectra with a JASCO Model J-20. TLC plates were made with silica gel (Kieselgel HF_{254}, Merck). Silica gel (Kieselgel 70-325 mesh, Merck) was used for column chromatography.

Isolation of Betulin(17), Lupeol(18) and Betulinaldehyde(19)——The rhizoma of *Scirpus fluviatilis* (dry wt., 4.8 kg), collected at Tatebayashi in Gumma prefecture in September, 1976, were pulverized and extracted with *n*-hexane (30 l×4) under reflux. The combined *n*-hexane extract was concentrated under reduced pressure to *ca.* 5 l. The precipitated crystalline substances were collected by filtration and recrystallized from $CHCl_3$–EtOH to give betulin (**17**) (21 g). The filtrate was concentrated under reduced pressure to give a brown mass (25.5 g). A part of this mass (15 g) was chromatographed on silica gel (300 g) developing with an ether-benzene mixture (5% ether in benzene→37% ether in benzene), successively (each fraction 300 ml). The fractions eluted with 5—9% ether in benzene (fr. No. 8—11) were combined and concentrated to yield a crystalline substance, which was purified by prep.-TLC (benzene) to give lupeol (**18**) (0.22 g) and betulinaldehyde (**19**) (0.24 g).

Betulin (**17**)——Colourless needles, mp 259—261°, $[\alpha]_D^{32}$ +18.2° (c=5.7, pyridine). MS, *m/e* (%): 442 (M^+, 19), 426 (6), 424 (9), 411 (38), 234 (26), 220 (23), 207 (68), 189 (100). *Anal.* Calcd. for $C_{30}H_{50}O_2 \cdot 1/2H_2O$: C, 79.76; H, 11.38. Found: C, 79.64; H, 11.26. This compound was identified with betulin by the direct comparison (IR and mixed mp).

Lupeol (**18**)——Colourless needles (from ether–*n*-hexane), mp 201—203°. *Anal.* Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.29; H, 11.80. Monoacetate, colourless needles (from EtOH), mp 220—223°, was identified with lupeol monoacetate by the direct comparison (IR and mixed mp).

Betulinaldehyde (**19**)[3]——Colourless needles (from ether–*n*-hexane), mp 150—154.5°. IR ν_{max}^{KBr} cm^{-1}: 3400, 3060, 2940, 2860, 2700, 1725, 1640, 880. ^{1}H NMR (δ in $CDCl_3$); 0.76 (3H), 0.83 (3H), 0.93 (3H), 0.96

13) O.R. Gottlieb, *Phytochemistry*, **11**, 1537 (1972); O.R. Gottlieb, J.G.S. Maia, and J.C. Mouraõ, *ibid*, **15**, 1289 (1976); W.D. Ollis and O.R. Gottlieb, *Chem. Commun.*, **1968**, 1396; J, Rouger and A. Robert, *Bull. Soc. Chim. France*, **1972**, 4039.

14) P. Coggon, T.J. King, and S.C. Wallwork, *Chem. Commun.*, **1966**, 439; *idem*, *J. Chem. Soc.*, B, **1970**, 884.

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(6H), 1.72 (3H) (each s, 6×CH_3), 4.65 (1H, br s), 4.76 (1H, br s) (=CH_2), 9.80 (1H, s, CHO). *Anal.* Calcd. for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98. Found: C, 81.90; H, 11.08. Monoacetate: colourless needles (from EtOH), mp 161—164°. *Anal.* Calcd. for: $C_{32}H_{50}O_3$: C, 79.62; H, 10.44. Found: C, 79.53; H, 10.49. **19** was reduced with $LiAlH_4$ in dry ether to give betulin (**17**) as colourless needles, mp 252—254° (identified by IR and mixed mp).

Isolation of Betulinic Acid (20), Resveratrol (15), 3,3′,4,5′-Tetrahydroxystilbene (16), Scirpusin A (1) and B (12)——The pulverized rhizoma of the plant, after extraction with *n*-hexane as mentioned above, were extracted with MeOH (30 l×5) under reflux. The combined MeOH extract was concentrated under reduced pressure to afford a dark brown mass (550 g), which was dissolved in H_2O (2 l) and extracted repeatedly with *n*-BuOH (total 8 l). The combined *n*-BuOH extract was concentrated under reduced pressure to afford a brown mass (140 g), which was extracted with hot $CHCl_3$. The $CHCl_3$ extract was concentrated and the residue (26 g) was subjected to silica gel column chromatography developing with $CHCl_3$ to yield betulinic acid (**20**) (0.13 g). The $CHCl_3$ insoluble part (103 g) of the *n*-BuOH extract was chromatographed on silica gel (1700 g) developing with benzene-ether mixture [benzene→benzene–ether (2: 8)] successively (each fr. 1000 ml), to yield resveratrol (**15**) [fr. No. 14—21, benzene–ether (6: 4), yield 0.81 g], 3,3′,4,5′-tetrahydroxystilbene (**16**) [fr. No. 22—30, benzene–ether (6: 4), 0.56 g] and the crude mixture of **1**, **12** and **16** [fr. No. 31—52, benzene–ether (5: 5—2: 8), 8.03 g]. The above crude mixture was purified by prep.-TLC [acetone–benzene (1: 1)] to give **16** (1.79 g), **1** (1.45 g) and **12** (1.64 g).

Betulinic Acid (20)——Colourless needles (from $CHCl_3$–EtOH), mp>300°. Methyl ester, colourless needles, mp 216—218°, was identified with betulinic acid methyl ester by the direct comparison (IR and mixed mp).

Resveratrol (15)[15]——Pale yellow powder (from acetone–benzene), mp 261°. UV λ_{max}^{EtOH} nm (log ε): 218 (4.33), 227 (sh 4.17), 307 (4.44), 320 (4.43). IR ν_{max}^{KBr} cm^{-1}: 3200—3300 (OH), 1630 (>C=C<), 1600, 1595, 1510 (aromatic), 965 (*trans* –CH=CH–). ^1H NMR (δ in d_6-acetone): 6.28 (1H, t, J=2 Hz, $C_{(4')}$–H), 6.55 (2H, d, J=2 Hz, $C_{(2')(6')}$–H), 6.76 (1H, d, J=17 Hz), 7.08 (1H, d, J=17 Hz) (*trans* –CH=CH–), 6.82 (2H, d, J=9 Hz), 7.40 (2H, d, J=9 Hz) (arom.-H, A_2B_2 type q), 8.23 (3H, br s 3×OH, D_2O exchangeable). *Anal.* Calcd. for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.32; H, 5.38. Triacetate: colourless needles (from EtOH–H_2O), mp 113.5—114.5°. ^1H NMR (δ in d_6-acetone): 2.23 (9H, s, 3×OAc), 6.83 (1H, t, J=2 Hz, $C_{(4')}$–H), 7.20—7.40 (4H, arom.–H and –CH=CH–), 7.08 (2H, d, J=9 Hz), 7.60 (2H, d, J=9 Hz) (arom.–H. A_2B_2 type q). *Anal.* Calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.86; H, 5.22. Trimethyl ether: colourless needles (from ether–*n*-hexane), mp 56—58°. ^1H NMR (δ in $CDCl_3$): 3.80 (9H, s, 3×OCH_3), 6.37 (1H, t, J=2 Hz, $C_{(4')}$–H), 6.66 (2H, d, J=2 Hz, $C_{(2')(6')}$–H), 6.95 (2H, s-like, –CH=CH–), 6.86 (2H, d, J= 9 Hz), 7.42 (2H, d, J=9 Hz) (arom.-H, A_2B_2 type q). *Anal.* Calcd. for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71. Found: C, 75.79; H, 6.78.

3,3′,4,5′-Tetrahydroxystilbene (16)[16]——Pale yellow needles (from acetone–benzene), mp 229°. UV λ_{max}^{EtOH} nm (log ε): 221 (4.37), 240 (sh 4.17), 293 (sh 4.21), 306 (sh 4.32), 326 (4.42). IR ν_{max}^{KBr} cm^{-1}: 3300, 1625, 1600, 1515, 1295, 1145, 960. ^1H NMR (δ in D_6-acetone): 6.28 (1H, t, J=2 Hz, $C_{(4')}$–H), 6.50 (2H, d, J= 2 Hz, $C_{(2')(6')}$–H), 6.8—7.2 (5H, 3×arom.-H and –CH=CH–), 8.06 (4H, br s, 4×OH, D_2O exchangeable). *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.84; H, 4.95. Found: C, 68.78; H, 5.03. Tetraacetate: colourless needles (from MeOH–H_2O), mp 116—117°. ^1H NMR (δ in $CDCl_3$): 2.27 (12H, s, 4×OAc), 6.83 (1H, t, J=2 Hz, $C_{(4')}$–H), 7.10 (2H, d, J=2 Hz, $C_{(2')(6')}$–H), 7.1—7.5 (5H, m, arom.-H and –CH=CH–). *Anal.* Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 63.97; H, 4.90. Tetramethyl ether: pale yellow powder, mp 63—65°. ^1H NMR (δ in d_6-acetone): 3.80 (9H, s, 3×OCH_3), 3.83 (3H, s, OCH_3), 6.35 (1H, t, J=2 Hz, $C_{(4')}$-H), 6.70 (2H, d, J=2 Hz, $C_{(2')(6')}$–H), 6.8—7.3 (5H, m, 3×arom.-H and –CH=CH–). *Anal.* Calcd. for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.69; H, 6.71.

Scirpusin A (1)——Pale brown amorphous powder, $FeCl_3$ in EtOH: dark green. UV λ_{max}^{EtOH} nm (log ε): 224 (sh 4.56), 290 (sh 4.27), 311 (sh 4.36), 323 (4.39). IR ν_{max}^{KBr} cm^{-1}: 3350 (br signal, OH), 1605, 1510 (aromatic and >C=C<), 960 (*trans* –CH=CH–). ^1H NMR (δ in d_6-acetone): 4.46 (1H, d, J=6 Hz, $C_{(3)}$–H), 5.40 (1H, d, J=6 Hz, $C_{(2)}$-H), 6.26 (3H, s, 3×arom.-H), 6.35 (1H, d, J=2 Hz, arom.-H), 6.5—7.3 (10H, m, arom.-H and –CH=CH–), 8.16 (6H, br s, OH, D_2O exchangeable). High resolution mass spectrum, Calcd. for $C_{28}H_{22}O_7$: 470.137. Found: 470.138.

Scirpusin A Hexamethyl Ether (2)——To a solution of **1** (361 mg) in ether (10 ml) was added ethereal diazomethane, and the solution was allowed to stand overnight and then concentrated to dryness. The residue was dissolved in DMF (2 ml) containing CH_3I (1 ml) and Ag_2O (200 mg), and stirred at room temperature for 20 hr. After filtration, the filtrate was poured into a large amount of water and extracted with ether for several times. The ethereal extracts were combined, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified by prep.-TLC [benzene–ether (4: 1)] to give an amorphous powder (**2**), 226 mg. UV λ_{max}^{EtOH} nm (log ε): 228 (sh 4.57), 290 (4.25), 310 (sh 4.39), 322 (4.42). IR ν_{max}^{KBr} cm^{-1}: 1605, 1600, 1510, 965. ^1H NMR (δ in d_6-acetone): 3.70 (6H, s), 3.73 (3H, s), 3.76 (6H, s), 3.80 (3H, s) (6×OCH_3), 4.65 (1H, d, J=6 Hz, $C_{(3)}$–H), 5.54 (1H, d, J=6 Hz, $C_{(2)}$–H), 6.3—7.3 (14H, m, arom.-H and –CH=CH–).

15) G. Nonaka, M. Minami, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2300 (1977).
16) F.E. King, T.J. King, D.H. Godson, and L.C. Manning, *J. Chem. Soc.*, **1956**, 4477; S.E. Drewes and I.P. Fletcher, *ibid.*, **1974**, 961.

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MS, *m/e* (%): 554 (M^+, 100), 403 (47), 257 (87), 165 (15),[17a] 151 (15).[17b] CD: $[\theta]^{23}_{400-250} \simeq 0$ ($c=0.00964$, MeOH). *Anal.* Calcd. for $C_{34}H_{34}O_7$: C, 73.63; H, 6.18. Found: C, 73.54; H, 6.22.

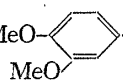
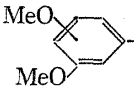
Scirpusin A Hexaacetate (3)——A solution of **1** (30 mg) in Ac_2O (1 ml) and pyridine (1 ml) was allowed to stand at room temperature overnight and then poured into ice-water. The resulted precipitates were collected and purified by prep.-TLC [benzene–ether (1: 1)] to give colourless needles (3), mp 174.5—176.5°, $[\alpha]_D^{31}$ 0° ($c=0.44$, $CHCl_3$), 24 mg. UV λ_{max}^{EtOH} nm (log ε): 214 (4.61), 292 (sh 4.48), 310 (4.49). IR ν_{max}^{KBr} cm^{-1}: 1760, 1615 (sh), 1610, 1585, 1500, 1370, 960. ^1H NMR (δ in d_6-acetone): 2.20, 2.23, 2.26 (each s, 18H, 6×OAc), 4.93 (1H, d, $J=6$ Hz, $C_{(3)}$–H), 5.72 (1H, d, $J=6$ Hz, $C_{(2)}$–H), 6.7—7.4 (14H, m, arom.-H and –CH=CH–). MS, *m/e* (%): 722 (M^+, 45), 680 (M^+-CH_2CO, 100), 638 ($680-CH_2CO$, 100), 596 ($638-CH_2CO$, 91), 554 ($596-CH_2CO$, 64), 512 ($554-CH_2CO$, 23), 215 (32), 137 (18), 123 (82). *Anal.* Calcd. for $C_{40}H_{34}O_{13}$: C, 66.48; H, 4.75. Found: C, 66.25; H, 4.66.

Oxidation of 2 by the Lemieux-Johnson's Method——To a solution of **2** (68 mg) in dioxane (6 ml) and H_2O (2 ml) was added OsO_4 (7 mg). The reaction mixture was stirred at room temperature for 15 min and then $NaIO_4$ (200 mg) were added to the mixture during 40 min with stirring. The reaction mixture was stirred for further 1 hr, filtered and extracted with ether. The ethereal extract was evaporated and purified by prep.-TLC [ether–*n*-hexane (1: 1)] to furnish anisaldehyde (14 mg) and **4** (45 mg). Anisaldehyde: colourless oil. ^1H NMR (δ in $CDCl_3$): 3.90 (3H, s, OCH_3), 7.00 (2H, d, $J=9$ Hz), 7.83 (2H, d, $J=9$ Hz) (A_2B_2 type q, arom. –H), 9.86 (1H, s, –CHO). 2,4-DNP-derivative, mp 252—257°, was identified with the authentic sample by the direct comparison (IR and mixed mp). **4**: amorphous powder, UV λ_{max}^{EtOH} nm (log ε): 277 (4.02), 346 (3.54). IR ν_{max}^{KBr} cm^{-1}: 1695, 1610, 1595, 1515. ^1H NMR (δ in d_6-acetone): 3.75 (6H, s), 3.80 (3H,s), 3.85 (3H, s), 3.92 (3H, s) (5×OCH_3), 4.98 (1H, d, $J=6$ Hz, $C_{(3)}$–H), 5.63 (1H, d, $J=6$ Hz, $C_{(2)}$–H), 6.42 (3H, s-like), 6.8—7.1 (5H, m) (8×arom.-H), 9.90 (1H, s, –CHO). MS, *m/e* (%): 450 (M^+, 100), 422 (M^+-CO, 9), 312 (29), 299 (47), 285 (11), 165 (11),[17a] 151 (22),[17b] 138 (16). *Anal.* Calcd. for $C_{26}H_{26}O_7$: C, 69.32; H, 5.82. Found: C, 69.36; H, 5.88.

Treatment of 4 with $LiAlH_4$——To a solution of **4** (110 mg) in dry ether (4 ml) was added $LiAlH_4$ (7 mg) and the mixture was stirred for 1 hr and then treated with H_2O. The precipitates were removed by filtration and the filtrate was concentrated. The residue was purified by prep.-TLC [acetone–benzene (1: 5)] to give **5**, colourless needles (from EtOH–H_2O), mp 124.5—127°, 95 mg. UV λ_{max}^{EtOH} nm (log ε): 234 (4.44), 278 (sh 3.80), 283 (3.84), 299 (sh 3.55). IR ν_{max}^{KBr} cm^{-1}: 3350, 1610, 1590, 1515. ^1H NMR (δ in d_6-acetone): 3.70 (6H, s), 3.73 (3H, s), 3.80 (6H, s) (5×OCH_3), 4.17 (2H, m, $-C\underline{H}_2OH$), 4.50 (1H, d, $J=6$ Hz, $C_{(3)}$–H), 5.45 (1H, d, $J=6$ Hz, $C_{(2)}$–H), 6.3—7.1 (8H, m, arom.-H). *Anal.* Calcd. for $C_{26}H_{28}O_7$: C, 69.01; H, 6.24. Found: C, 69.25; H, 6.22.

Treatment of 5 with *p*-Bromobenzoyl Chloride——To a solution of **5** (53 mg) in dry acetone containing K_2CO_3 (200 mg) was added *p*-bromobenzoyl chloride (50 mg). The reaction mixture was refluxed for 4 hr, filtered and concentrated. The residue was purified by prep.-TLC [acetone–benzene (1: 5)] to furnish **6**, colourless needles (from ether–*n*-hexane), mp 104.5—106°, 32 mg. UV λ_{max}^{EtOH} nm (log ε): 235 (4.54), 277 (sh, 3.91), 283 (3.96), 297 (sh 3.68). IR ν_{max}^{KBr} cm^{-1}: 1720, 1610, 1590, 1520. ^1H NMR (δ in $CDCl_3$): 3.65 (6H, s), 3.78 (6H, s), 3.83 (3H, s), (5×OCH_3), 4.45 (1H, d, $J=6$ Hz, $C_{(3)}$–H), 4.90 (2H, br s, $-CH_2O$), 5.46 (1H, d, $J=6$ Hz, $C_{(2)}$–H), 6.25 (3H, s), 6.52 (2H, br s), 6.82 (3H, s) (8×arom.-H), 7.46 (2H, d, $J=9$ Hz), 7.73 (2H, d, $J=9$ Hz) (A_2B_2 type q, *p*-Br–$C_6\underline{H}_4CO$–). *Anal.* Calcd. for $C_{33}H_{31}BrO_9$: C, 62.36; H, 4.92. Found: C, 62.23; H, 4.96.

Catalytic Hydrogenation of 5 with Pd-C/H_2 in MeOH——**5** (61 mg) was dissolved in MeOH (15 ml) containing AcOH (0.5 ml) and shaken with H_2 in the presence of Pd–C as a catalyst (overnight). The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was purified by prep.-TLC [acetone–benzene (1: 5)] to give **7** (16 mg) and **8** (28 mg). **7**: *Rf* 0.57, colourless needles (from ether–*n*-hexane), mp 101.5—102.5°. UV λ_{max}^{EtOH} nm (log ε): 209 (4.96), 230 (sh 4.46), 279 (sh 3.91), 282 (3.98), 289 (sh 3.83). IR ν_{max}^{KBr} cm^{-1}: 1605, 1595, 1515. ^1H NMR (δ in $CDCl_3$): 1.86 (3H, s, arom.-CH_3), 3.70 (6H, s), 3.75 (3H, s), 3.80 (3H, s), 3.84 (3H, s) (5×OCH_3), 4.33 (1H, d, $J=6$ Hz, $C_{(3)}$–H), 5.42 (1H, d, $J=6$ Hz, $C_{(2)}$–H), 6.2—6.5 (5H, m), 6.80 (3H, s) (8×arom.-H). *Anal.* Calcd. for $C_{26}H_{28}O_6$: C, 71.54; H, 6.47. Found: C, 71.75; H, 6.47. **8**: *Rf* 0.40, colourless prisms (from ether–*n*-hexane), mp 111.5—113.5°. Gibbs test: deep blue; $FeCl_3$ in EtOH: brown. UV λ_{max}^{EtOH} nm (log ε): 209 (4.88), 230 (sh 4.44), 276 (sh 3.85), 279 (3.86), 286 (sh 3.75). IR ν_{max}^{KBr} cm^{-1}: 3430 (strong peak, OH), 1600, 1590, 1510. ^1H NMR (δ in $CDCl_3$): 1.83 (3H, s, arom.-CH_3), 3.2—3.6 (2H, m, $-CH_2-$), 3.57 (3H, s), 3.70 (3H, s), 3.75 (6H, s), 3.80 (3H, s) (5×OCH_3), 4.43 (1H, m, –CH), 4.90 (1H, s, OH, D_2O exchangeable), 6.2—6.7 (8H, m, arom.-H). *Anal.* Calcd. for $C_{26}H_{30}O_6$: C, 71.21; H, 6.90. Found: C, 71.34; H, 6.93. **8** (36 mg) was methylated with $(CH_3)_2SO_4$ and K_2CO_3 in acetone under reflux to afford **9** as an amorphous powder (30 mg). ^1H NMR (δ in $CDCl_3$): 2.00 (3H, s, arom.-CH_3), 3.38 (2H, d, $J=8$ Hz, $-CH_2-$), 3.63 (3H, s), 3.65 (3H, s), 3.70 (3H, s), 3.75 (6H, s), 3.80 (3H, s) (6×OCH_3), 4.48 (1H, t, $J=8$ Hz, –CH), 6.1—6.7 (8H, m, arom.-H).

17) *a*) *m/e*, 165=MeO-  -C≡O$^+$ *b*) 151=  -CH_2 .

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Treatment of 9 with DDQ——A solution of **9** (30 mg) in dry benzene (4 ml) containing DDQ (20 mg) was refluxed for 2 hr and then filtered. The filtrate was concentrated and purified by prep.-TLC [acetone–benzene (1: 10)] to give **10** (10 mg) and **11** (17 mg). **10**: *Rf* 0.55, colourless needles (from ether–*n*-hexane), mp 127—129°. UV λ_{max}^{EtOH} nm (log ε): 242 (sh 4.40), 285 (4.20), 299 (sh 4.17). IR ν_{max}^{KBr} cm^{-1}: 1600, 1590, 1510. ^{1}H NMR (δ in $CDCl_3$): 2.28 (3H, s arom.-CH_3), 3.55 (3H, s), 3.60 (6H, s), 3.65 (3H, s), 3.76 (3H, s), 3.80 (3H, s) (6×OCH_3), 6.2—6.5 (6H, m), 6.70 (3H, s), (8×arom.-H and =CH). MS, *m/e* (%): 450 (M^+, 25), 435 (M^+-CH_3, 1), 255 (12.5), 255 (12), 202 (10), 196 (12), 191 (10), 183 (32.5), 164 (18), 162 (14), 152 (18), 151 (28), 132 (16), 128 (12), 105 (100). *Anal.* Calcd. for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 71.53; H, 6.66. **11**: *Rf* 0.52, amorphous powder. UV λ_{max}^{EtOH} nm (log ε): 291 (sh 4.23), 321 (4.35). IR ν_{max}^{KBr} cm^{-1}: 1600, 1590 (sh), 1510. ^{1}H NMR (δ in $CDCl_3$): 2.00 (3H, s, arom.-CH_3), 3.43 (3H, s), 3.55 (3H, s), 3.72 (6H, s), 3.77 (6H, s), (6×OCH_3), 6.3—6.8 (8H, m, arom.-H), 7.10 (=CH). MS, *m/e* (%): 450 (M^+, 100), 435 (M^+-CH_3, 4), 225 (4), 196 (4), 183 (12), 152 (4), 151 (6), 149 (4), 132 (5), 105 (19).

Ozonolysis of 10 and 11——Ozonized O_2 was passed through a solution of **10** and **11** (27 mg as mixture) in $CHCl_3$ (5 ml) at −70° for 1 min and then H_2O (0.5 ml) was added to the reaction mixture. The mixture was stirred at room temperature for 30 min, concentrated under reduced pressure and purified by prep.-TLC [ether–*n*-hexane (1: 1)] to give veratraldehyde as a colourless oil, 2.5 mg. ^{1}H NMR (δ in $CDCl_3$): 3.93 (3H, s), 3.96 (3H, s), (2×OCH_3), 6.96 (1H, d, J=9 Hz), 7.38 (1H, s-like), 7.45 (1H, J=9/2 Hz), (3×arom.-H), 9.82 (1H, s, -CHO).[18] Its 2,4-DNP-derivative, reddish prisms, mp 265—268°, was identified with 2,4-DNP-derivative of veratraldehyde by the direct comparison (IR and mixed mp).

Scirpusin B (12)——Pale brown amorphous powder, $FeCl_3$ in EtOH: dark green. UV λ_{max}^{EtOH} nm (log ε): 225 (sh 4.55), 290 (4.17), 308 (sh 4.21), 331 (4.32). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1605, 1515 (aromatic and olefinic), 960 (*trans* -CH=CH-). ^{1}H NMR (δ in d_6-acetone): 4.45 (1H, d, J=6 Hz, $C_{(3)}$-H), 5.37 (1H, d, J=6 Hz, $C_{(2)}$-H), 6.26 (3H, s), 6.33 (1H, d, J=2 Hz), 6.7—7.0 (9H, m) (13H, arom.-H and -CH=CH-), 8.10 (7H, br s, OH, D_2O exchangeable). MS, *m/e* (%): 486 (M^+, $C_{28}H_{22}O_8$, 5), 378 (100), 244 (38), 242 (41).

Scirpusin B Heptaacetate (13)——A solution of **12** (100 mg) in Ac_2O (1 ml) and pyridine (1 ml) was allowed to stand at room temperature overnight and then poured into ice-water. The resulted precipitates were collected and purified by prep.-TLC [benzene–ether (1: 1)] to give **13**, colourless needles (from EtOH–H_2O), mp 97—102°, 50 mg. UV λ_{max}^{EtOH} nm (log ε): 303 (sh 4.33), 310 (4.34). IR ν_{max}^{KBr} cm^{-1}: 1760, 1610, 1585, 1500, 960. ^{1}H NMR (δ in d_6-acetone): 2.20—2.27 (21H, 7×OAc), 4.93 (1H, d, J=6 Hz, $C_{(3)}$-H), 5.70 (1H, d, J=6 Hz, $C_{(2)}$-H), 6.9—7.2 (9H, m), 7.30 (3H, s) (13H, arom.-H and -CH=CH-). MS, *m/e* (%): 780 (M^+, 18), 738 (M^+-CH_2CO, 36), 696 (738−CH_2CO, 100), 654 (696−CH_2CO, 71), 612 (654−CH_2CO, 46), 570 (612−CH_2CO, 25), 528 (570−CH_2CO, 14), 486 (528−CH_2CO, 7). CD: $[\theta]_{400-250}^{25}$=0 (c=0.041, dioxane). *Anal.* Calcd. for $C_{42}H_{36}O_{15}$: C, 64.61; H, 4.65. Found: C, 64.36; H, 4.67.

Scirpusin B Heptamethyl Ether (14)——**12** (215 mg) was methylated by the same procedure as in the case of **1** to give **14**, amorphous powder, 121 mg. UV λ_{max}^{EtOH} nm (log ε): 228 (sh 4.67), 286 (4.22), 308 (sh 4.28), 330 (4.40), 350 (sh 4.19). IR ν_{max}^{KBr} cm^{-1}: 1605, 1590, 1510, 960. ^{1}H NMR (δ in $CDCl_3$): 3.67 (6H, s), 3.74 (3H, s), 3.78 (3H, s), 3.83 (9H, s) (7×OCH_3), 4.50 (1H, d, J=6 Hz, $C_{(3)}$-H), 5.50 (1H, d, J=6 Hz, $C_{(2)}$-H), 6.3—7.1 (13H, m, arom.-H and -CH=CH-); ^{1}H NMR (δ in d_6-acetone): 3.67 (9H, s), 3.70 (6H, s), 3.72 (3H, s), 3.78 (3H, s) (7×OCH_3), 4.60 (1H, d, J=6 Hz, $C_{(3)}$-H), 5.46 (1H, d, J=6 Hz, $C_{(2)}$-H), 6.30—6.46 (4H, m), 6.64—6.96 (7H, m) (arom.-H), 6.61 (1H, d, J=16 Hz), 6.95 (1H, d, J=16 Hz) [*trans* -CH=CH-, these signals were assigned by double resonance experiment; irradiation at δ 6.95→δ 6.61 (doublet→singlet)]. *Anal.* Calcd. for $C_{35}H_{36}O_8$: C, 71.90; H, 6.21. Found: C, 71.80; H, 6.21.

Oxidation of 14 by the Lemieux-Johnson's Method——A solution of **14** (94 mg) in a mixture of dioxane (6 ml) and H_2O (2 ml) containing OsO_4 (7 mg) was stirred at room temperature for 15 min and then $NaIO_4$ (200 mg) were added to the reaction mixture during 40 min with stirring. The mixture was stirred at room temperature for further 1 hr and filtered. The filtrate was treated with H_2O and extracted with ether for several times. The combined ethereal extract was concentrated and purified by prep.-TLC [ether–*n*-hexane (2: 1)] to give veratraldehyde (22 mg) and **4** (57 mg). Veratraldehyde: colourless oil. ^{1}H NMR (δ in $CDCl_3$): 3.90 (3H, s), 3.93 (3H, s) (2×OCH_3), 7.10 (1H, d, J=9 Hz), 7.40 (1H, s-like), 7.53 (1H, d,d. J=9/2 Hz) (3×arom.-H), 9.82 (1H, s, -CHO).[18] The 2,4-DNP-derivative, reddish orange prisms, mp 265—268°, was identified with the authentic sample by the direct comparison (IR and mixed mp). **4** was identified by the comparison of IR and ^{1}H NMR spectra with **4** prepared from **2** as mentioned above.

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18) N.S. Bhacca, D.P. Hollis, L.F. Johnson, E.A. Pier, and J.N. Shoolery, "High Resolution NMR Spectra Catalog" by Varian Associates, National Press, 1962, 236.

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