liquors of the crystallization ($CHCl_3$) of fraction *e*. It had mp 131–132° (from $CHCl_3$), $[\alpha]_D$ −71° (dioxane; *c* 0.6). UV λ_{max}^{MeOH} nm (log ε): 288 (4.25). EIMS, 70 eV, *m/z* (rel. int.): 346.1048 ($[M]^+$; calc. for $C_{18}H_{18}O_7$: 346.1052) (20), 107 (100).

Compound **2** (20 mg) was isolated by TLC [C_6H_6–EtOAc (23:2), 3 runs] of the crystals obtained by crystallization ($CHCl_3$) of fraction *b*. It had mp 136–138° (from $CHCl_3$), $[\alpha]_D$ −51° (MeOH; *c* 0.7). UV λ_{max}^{MeOH} nm (log ε): 287 (4.38). EIMS, 70 eV, *m/z* (rel. int.): 316.0952 ($[M]^+$; calc. for $C_{17}H_{16}O_6$: 316.0947) (25), 137 (100).

Compound **3** (20 mg) was obtained by TLC [C_6H_6–EtOAc (23:2), 3 runs] from the mother liquors of the crystallization ($CHCl_3$) of fraction *c*. It had mp 103–104° (from C_6H_6–MeOH), $[\alpha]_D$ −34° (MeOH; *c* 0.4) UV λ_{max}^{MeOH} nm (log ε): 287 (4.22). EIMS, eV, *m/z* (rel. int.): 286.0849 ($[M]^+$, calc. for $C_{16}H_{14}O_5$. 286.0841) (20), 107 (100).

Acknowledgements—This work was supported by the Ministero della pubblica Istruzione. NMR spectra were performed at the Centro di Metodologie Chimico-Fisiche della Università di Napoli.

REFERENCES

1. Adinolfi, M., Barone, G., Belardini, M., Lanzetta, R., Laonigro, G. and Parrilli, M. (1984) *Phytochemistry* **23**, 2091.
2. Heller, W. and Tamm, C. (1981) *Fortschr. Chem. Org. Naturst.* **40**, 105.
3. Markham, K. R. and Mabry, T. J. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.), p. 45. Chapman & Hall, London.
4. Schaad, W. (1977) Ph.D. Thesis, Basel.

Phytochemistry, Vol. 24, No. 3, pp. 626–628, 1985.
Printed in Great Britain.

0031-9422/85 $3.00 + 0.00

LIGNAN FROM CALYCES OF *DIOSPYROS KAKI*

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(*Received* 20 *June* 1984)

Key Word Index—*Diospyros kaki*; Ebenaceae, calyx, (−)-divanillyltetrahydrofuran ferulate.

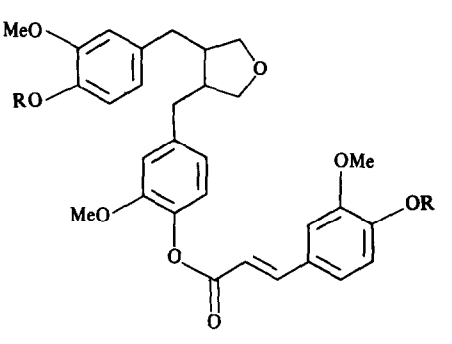
Abstract—From *Diospyros kaki* calyces, a new lignan was isolated. Its structure was elucidated as (−)-divanillyltetrahydrofuran ferulate by spectroscopic methods and was established by total synthesis.

INTRODUCTION

Calyces of *Diospyros kaki* are used both in Japanese folk medicine and in traditional Chinese medicine for the treatment of hiccough. In previous papers [1, 2], as the constituents of the calyces, 18 compounds comprising flavonols and their glycosides, triterpenoids and aromatic acids were isolated and their structures determined. The present paper deals with the structural elucidation of a new lignan and its confirmation by total synthesis.

RESULTS AND DISCUSSION

Compound **1** (R = H) was isolated from the crude acetone extract of the calyces of *D. kaki* by repeated column chromatography on silica gel, mp 184–185°, $[\alpha]_D$ −58.3° (THF), as colourless needles, and formed a diacetate ($[M]^+$ 622) and a di-*O*-methyl ether (**2**) (R = Me) ($[M]^+$ 548), respectively. The mass spectrum of **1** ($[M]^+$ 520) and elemental analysis indicated that it had the molecular formula $C_{30}H_{32}O_8$. In particular, the presence of ions at *m/z* 137 and 138 is characteristic of the vanillyl group. In the ^1H NMR spectrum, the two-proton signal at δ2.15 and the four-proton one at δ2.74 confirmed a tetrahydrofuran ring, which was further confirmed by the signals at δ63.7 (triplet) and 39.4 (doublet) in the



1 R = H
2 R = Me

^{13}C NMR spectrum. A set of signals comprising a one-proton doublet at δ6.55 and 7.62 (J = 16 Hz) in the ^1H NMR spectrum suggested the presence of a cinnamoyl derivative. The IR absorption bands at 1700 and 1257 cm^{-1} suggested that **1** contained an α,β-unsaturated acid as ester. Hydrolysis of **1** in ethanol containing hydrogen chloride gave 3,4-bis[4-hydroxy-3-methoxybenzyl]-tetrahydrofuran (**3**), mp 110–111° (lit. mp 116–117°, $[\alpha]_D$ − 52.2° [3]) and ferulic acid, mp 172–174°. From the spectral data described above, the structure for **1** was concluded to be (−)-divanillyltetrahydrofuran ferulate.

In order to confirm the structure, **2** and **3** were prepared synthetically by the method of Batterbee *et al.* [4]. Stobbe condensation of vanillin benzyl ether (**4**) with diethyl succinate gave the benzylidene half-ester (**5**), which was further condensed with **4** after methyl esterification to afford dibenzylidenesuccinic acid (**6**). Compound **6** was hydrogenated with Pd–C in an autoclave to give the debenzylated tetrahydro derivative (**7**). Reduction of **7** with lithium aluminium hydride followed by cyclization with *p*-tosyl chloride gave **3**, mp 112–113°, which was identical (mp, co-TLC and IR) to the sample obtained by hydrolysis of **1**. On the other hand, condensation of **5** with veratraldehyde, followed by the same procedures described above gave 3-(3,4-dimethoxybenzyl)-4-(4-hydroxy-3-methoxybenzyl)-tetrahydrofuran (**8**). Esterification of **8** with 3,4-dimethoxycinnamic acid in the presence of dry trifluoroacetic acid gave **2**, mp 145–147°, which was identical (mp and co-TLC) to the di-*O*-methyl ether of **1**. The structure of **1** could thus be established as (−)-divanillyltetrahydrofuran ferulate.

EXPERIMENTAL

Plant material. The plant breeding of *D. kaki* is called '*Hachiya*' in Japan. The procedures of extraction and separation of constituents have been described in detail in previous papers [1, 2].

Isolation of compound **1**. Fractions on silica gel chromatography (eluant $CHCl_3$) showing a TLC spot, R_f 0.20 EtOAc–C_6H_{14}, 1.1), were concd to afford **1** (220 mg). Recrystallization from EtOAc gave colourless needles, mp 184–185°. $[\alpha]_D^{25}$ − 58.3° (THF; *c* 0.12). (Found: C, 66.84; H, 6.27. $C_{30}H_{32}O_8 \cdot H_2O$ requires: C, 66.90; H, 6.36%.) MS *m/z* (rel. int.): 520 $[M]^+$ (4), 344 (23), 326 (14), 194 (12), 189 (20), 177 (32), 150 (16), 138 (53), 137 (100). UV λ_{max}^{MeOH} nm (log ε): 233 (4.3), 290 (4.2), 327 (4.3). IR ν_{max}^{KBr} cm^{-1}: 3390, 1700, 1610, 1515, 1257, 1218 ^1H NMR (80 MHz, DMSO-d_6, TMS): δ2.15 (2H, *m*, $CH_2C\underline{H}CH_2O$), 2.74 (4H, *m*, $ArC\underline{H}_2$), 3.73 (3H, *s*, OMe), 3.86 (6H, *s*, 2 × OMe), 4.22 (4H, *m*, $CHC\underline{H}_2O$), 6.55 (1H, *d*, J = 16 Hz, $C\underline{H}$=CHCO), 6.61–7.38 (9H, *m*, ArH), 7.62 (1H, *d*, J = 16 Hz, CH=$C\underline{H}CO_2$), 8.78 (2H, *br s*, 2 × OH), 9.67 (1H, *br s*, OH). ^{13}C NMR (25.05 MHz, DMSO-d_6, TMS): δ166.6 (*s*), 149.3 (*s*), 147.8 (*s*), 147.3 (*s*), 145.0 (*d*), 144.6 (*s*), 130.7 (*s*), 125.5 (*s*), 123.0 (*d*), 121.0 (*d*), 115.4 (*d* and *s*), 114.3 (*d*), 112.7 (*d*), 111.1 (*d*), 63.7 (*t*, $CH\underline{C}H_2O$), 55.6 (*q*), 55.3 (*q*), 39.4 (*d*, $CH_2\underline{C}HCH_2$), 33.7 (*t*, $Ar\underline{C}H_2$).

Methylation of **1**. A soln of **1** (30 mg) in MeOH was treated with excess CH_2N_2–Et_2O at 5° for 12 hr. The crystalline material obtained from Et_2O upon evapn was recrystallized to afford **2** (25 mg), mp 146–147°, as colourless needles.

Acetylation of **1**. Compound **1** (20 mg) was allowed to react with Ac_2O (2 ml) in pyridine (2 ml) overnight. Usual work-up gave the diacetate as an oil. MS *m/z*: 622 $[M]^+$. ^1H NMR ($CDCl_3$): δ2.81, 2.82 (each 3H, *s*, OAc).

Hydrolysis of **1**. Compound **1** (60 mg) was treated with 10% HCl–EtOH under reflux for 4 hr. Usual work-up afforded ferulic acid (mp 172–174°) and **3** (25 mg). Recrystallization of **3** from C_6H_6 gave colourless needles, mp 110–111° (lit mp 116–117° [3]) (Found: C, 69 69; H, 7.05. $C_{20}H_{24}O_5$ requires: C, 69.75; H, 7.02%). MS *m/z* (rel. int.): 344 $[M]^+$ (36), 189 (7), 138 (89), 137 (100). UV λ_{max}^{MeOH} nm (log ε): 231 (4.1), 280 (3.8) ^1H NMR ($CDCl_3$): δ2.18 (2H, *m*, $C\underline{H}CH_2O$), 2.57 (4H, *d*, J = 7 Hz, $C\underline{H}_2CH$), 3.41–4.07 (4H, *m*, CH_2O), 3.84 (6H, *s*, 2 × OMe), 6.54 (2H, *d*, J = 1.5 Hz, H-2, H-2'), 6.61 (2H, *dd*, J = 8, 1.5 Hz, H-6, H-6'), 6.85 (2H, J = 8 Hz, H-5, H-5') [5]. Ferulic acid was confirmed by comparison with an authentic sample and with one of isoferulic acid.

Synthesis of (±)-*divanillyltetrahydrofuran.* (a) According to ref. [4], 4-benzyloxy-3-methoxybenzylidenesuccinic acid (**5**) (66 g) was obtained by the condensation of diethyl succinate (34.8 g) with vanillin benzyl ether (**4**) (48.4 g), mp 174–175°. MS *m/z* (rel. int.): 342 $[M]^+$ (7), 298 (5), 207 (7), 151 (2), 91 (100). The dimethyl ester (prepared using Me_2SO_4, K_2CO_3, DMF) had mp 78–81° (from EtOAc–C_6H_{14}) ^1H NMR ($CDCl_3$): δ3.42 (2H, *s*, $C\underline{H}_2COOMe$), 7.61 (1H, *s*, CH=C). MS *m/z* (rel. int.): 370 $[M]^+$ (2), 356 (53), 324 (2), 265 (6), 205 (10), 123 (14), 91 (100). The ester (37 g) was condensed with **4** (24 g) to give di-(4-benzyloxy-3-methoxy)-benzylidenesuccinic acid (49 g), mp 164–166°, IR ν_{max}^{KBr} cm^{-1}: 1670 (C=O). ^1H NMR ($CDCl_3$): δ7.83 (2H, *s*, CH=C). The dimethyl ester (prepared using MeI, K_2CO_3 and DMF) had mp 142–144° (from $CHCl_3$) (Found. C, 72.42, H, 5.81. $C_{36}H_{34}O_8$ requires: C, 72.21; H, 5.76%). IR ν_{max}^{KBr} cm^{-1}. 1705 (C=O). MS *m/z* (rel. int.): 594 $[M]^+$ (86), 503 (27), 471 (100), 443 (29), 399 (27), 402 (23), 321 (34), 307 (25), 227 (18). ^1H NMR ($CDCl_3$): δ3 61 (6H, *s*, 2 × COOMe), 3.68 (6H, *s*, 2 × OMe), 5.01 (4H, *s*, 2 × $OC\underline{H}_2Ph$), 6.66 (2H, *d*, J = 7 Hz, H-5, H-5'), 6.95 (2H, *dd*, J = 7, 2 Hz, H-6, H-6'), 7 01 (2H, *d*, J = 2, H-2, H-2'), 7 23 (10H, *s*, 2 $OCH_2\underline{Ph}$), 7 73 (2H, *s*, CH=C). (b) The above ester (20 g) in EtOAc (200 ml) was hydrogenated over Pd–C (10%, 2 g) in an autoclave (50 atm, 80°, 8 hr) to give the tetrahydro derivative (**7**), followed by debenzylation, as colourless needles (8 g) from C_6H_6, mp 140–142° (Found: C, 63.21; H, 6.28. $C_{22}H_{26}O_8$ requires. C, 63.15; H, 6.26%). ^1H NMR ($CDCl_3$): δ2.91 (6H, *br s*, 2 × $ArC\underline{H}_2C\underline{H}$). MS *m/z* (rel. int.): 418 $[M]^+$ (12), 209 (16), 177 (16), 138 (11), 137 (100), 124 (38). (c) Usual reduction of **7** (2 g) with $LiAlH_4$ gave the diol, mp 115–118° (from C_6H_6), 1.1 g. ^1H NMR ($CDCl_3$): δ2.0 (2H, *m*, 2 × $ArCH_2C\underline{H}$), 2.64 (4H, *m*, 2 × $C\underline{H}_2Ar$), 4.12 (4H, *m*, 2 × $C\underline{H}_2OH$). MS *m/z* (rel. int.): 362 $[M]^+$ (11), 344 (5), 189 (8), 153 (6), 138 (31), 137 (100). (d) The above diol (800 mg) was treated with *p*-tosyl chloride (600 mg) to give **3** as colourless needles, mp 112–113° (from C_6H_6–C_6H_{14}), IR ν_{max}^{KBr} cm^{-1}: 3310, 2920, 1600, 1505, 1430. MS *m/z* (rel int.): 344 $[M]^+$ (45), 186 (6), 151 (12), 138 (99), 137 (100)

Synthesis of (±)-3-(3,4-*dimethoxybenzyl*)-4-[4-(3,4-*dimethoxycinnamoyloxy*)-3-*methoxybenzyl*]*tetrahydrofuran* (**9**). (a) By the same method described above, **4** (36.8 g) was condensed with veratraldehyde (16.6 g) to give α-(3,4-dimethoxybenzylidene)-β-(4-benzyloxy-3-methoxybenzylidene)succinic acid (**10**) (50 g) as pale yellow prisms, mp 197–198° (from C_6H_6). ^1H NMR (DMSO-d_6): δ7.69 (2H, *s*, 2 × CH=C). After methylation, the resulting diester (45 g) was hydrogenated to give the tetrahydro derivative (**11**) as colourless needles (18 g), mp 135–137° (from MeOH) (Found: C, 63.76; H, 6.53. $C_{23}H_{28}O_8$ requires: C, 63.88; H, 6.58%). MS *m/z* (rel. int.): 432 $[M]^+$ (45), 401 (3), 369 (2), 223 (12), 191 (17), 177 (9), 151 (100), 138 (40), 137 (52), 223 (20). (b) Reduction of **11** (16.5 g) with $LiAlH_4$ gave the diol as colourless prisms (12.1 g), mp 116–118° (from EtOAc) (Found: C, 67.12, H, 7.72. $C_{21}H_{28}O_6$ requires: C, 67.00; H, 7.50%). IR ν_{max}^{KBr} cm^{-1}: 3480, 2940, 1600, 1515, 1475, 1280, 1250 MS *m/z*

(rel. int.): 376 $[M]^+$ (43), 358 (5), 203 (7), 189 (9), 177 (10), 152 (42), 151 (100), 138 (22), 137 (96). ^1H NMR ($CDCl_3$): δ1.87 (2H, *m*, 2 × CHCH$_2$OH), 2.70 (4H, *m*, 2 × ArCH$_2$), 3.57–3.90 (4H, *m*, 2 × CH$_2$OH). Upon treatment with *p*-tosyl chloride, the diol (4 g) gave **8** as colourless prisms (2.1 g), mp 103–105°. MS *m/z* (rel. int.): 358 $[M]^+$ (67), 203 (5), 189 (6), 177 (7), 152 (67), 151 (100), 138 (37), 137 (64). (c) To C_6H_6 (30 ml) containing $(CF_3CO)_2O$ (1 ml) and 3,4-dimethoxycinnamic acid (1.0 g), **8** (1.8 g) was added. The mixture was stirred for 13 hr at room temp. and poured into H_2O. The soln was extracted with EtOAc and the EtOAc was evapd under red. pres. to give crystalline material. Recrystallization from MeOH gave **9** as colourless needles, mp 145–147° (Found: C, 70.20; H, 6.84. $C_{32}H_{36}O_8$ requires: C, 70.05; H, 6.61%). MS *m/z* (rel. int.): 548 $[M]^+$ (4), 504 (1), 358 (2), 192 (13), 191 (100), 151 (11). UV λ_{max}^{MeOH} nm (log ε): 230 (4.2), 288 (4.1), 324 (4.2). IR ν_{max}^{KBr} cm^{-1}: 2920, 1700, 1620, 1600, 1510. ^1H NMR ($CDCl_3$): δ2.40–2.80 (6H, *m*, 2 × ArCH$_2$CH), 3.82 (6H, *s*, 2 × OMe), 3.89 (9H, *s*, 3 × OMe), 3.51–3.90 (4H, *s*, 2 × CH$_2$O), 6.48 (1H, *d*, *J* = 16 Hz, CH=CHCO$_2$), 6.61–7.24 (9H, *m*, ArH), 7.75 (1H, *d*, *J* = 16 Hz, CH=CHCO$_2$). Compound **9** was identical (mp, co-TLC) to **2**.

REFERENCES

1. Matsuura, S. and Iinuma, M. (1977) *Yakugaku Zasshi* **97**, 452.
2. Matsuura, S. and Iinuma, M. (1978) *Chem. Pharm. Bull.* **26**, 1936.
3. Freudenberg, K. and Knof, L. (1957) *Chem. Ber.* **90**, 2857.
4. Batterbee, J. E., Burden, R. S., Crombie, L. and Whiting, D. A. (1969) *J. Chem. Soc. C* 2470.
5. Modonova, L. D., Voronov, V. K., Leont'eva, V. G. and Tyukavkina, N. A. (1972) *Khim. Prir. Soedin.* 165.

Phytochemistry, Vol. 24, No. 3, pp 628–630, 1985
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

A LIGNAN FROM *LONICERA HYPOLEUCA**

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(*Revised received* 6 *September* 1984)

Key Word Index—*Lonicera hypoleuca*; Caprifoliaceae; lignan; 4-hydroxy-2,6-di-(4'-hydroxy-3'-methoxy)phenyl-3,7-dioxabicyclo(3.3.0)octane; *n*-10-nonacosanol; scopoletin; syringic acid; sitosterol; β-sitosterol-β-D-glucoside; spasmolytic activity.

Abstract—A new lignan characterised as (−)-4-hydroxy-2,6-di-(4'-hydroxy-3'-methoxy)phenyl-3,7-dioxabicyclo-(3.3.0)octane along with *n*-10-nonacosanol, scopoletin, syringic acid, β-sitosterol and its glucoside, has been isolated from the aerial parts of *Lonicera hypoleuca*. The stereochemistry of the lignan has been established by its spectroscopic analysis and those of its derivatives, and by its conversion to (+)-pinoresinol. β-Sitosterol-β-D-glucoside displayed good spasmolytic activity.

INTRODUCTION

In continuation of the efforts aimed at the development of drugs from natural sources [1–3], a 50% aqueous ethanol extract of the aerial parts of *Lonicera hypoleuca* was found to exhibit spasmolytic activity in guinea-pig ileum. From the chloroform-soluble fraction of the alcoholic residue a new lignan has been isolated whose structural elucidation is described in the present communication.

RESULTS AND DISCUSSION

Compound **1**, mp 152° ($CHCl_3$), $[\alpha]_D^{25}$ −44° (MeOH), $C_{20}H_{22}O_7$, $[M]^+$ *m/z* 374.14004, exhibited the signals in its ^1H NMR spectrum for the presence of (a) two symmetrically substituted dioxygenated phenyl rings, (b) two magnetically non-equivalent benzylic methines situated at carbons bearing oxygen functions, (c) one hemiacetal proton almost identical in situation to that of 4-hydroxysesamin **6** [4], and (d) two OCH_3 groups. In addition, it contained signals for two non-equivalent methylene and two upfield methine protons. **1** furnished tetra-acetate **2**, tetramethoxy **3** and pentamethoxy **4** derivatives (Table 1) which confirmed the presence of two phenolic and one hemiacetal OH functions in **1**; the remaining two oxygen atoms being part of the heterocyclic rings. The double resonance experiments in **2** confirmed the vicinality of one of the upfield methines (δ3.21) to the methylene (δ4.02, 4.24) on one hand and to one of the benzylic methines (δ5.10) on the other. Likewise, the other upfield methine (δ3.03) was found to be situated in between the other benzylic methine (δ4.92) and the hemiacetal methine (δ6.4). The above data indicated **1** to be a lignan belonging to diarylfurofuran group and was further supported by

*CDRI Communication No. 3552.