(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**

KHALID A. KHAN and ABOO SHOEB

Central Drug Research Institute, Lucknow 226 001, India

(*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

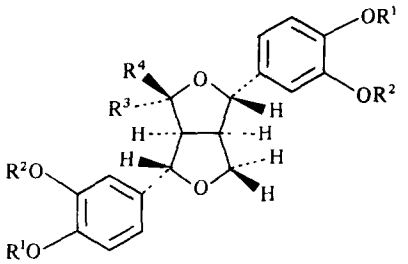
Compound **1**, mp 152° ($CHCl_3$), $[\alpha]_D^{25}$ −44° (MeOH), $C_{20}H_{22}O_7$, $[M]^+$ *m/z* 374.14004, exhibited the signals in its ^1H NMR spectrum for the presence of (a) two symmetrically substituted dioxygenated phenyl rings, (b) two magnetically non-equivalent benzylic methines situated 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.

Table 1

Protons	Chemical shift, δ(ppm) Solvent, $CDCl_3$ 1	2	3	4	J value (Hz) in parentheses 6
H-1	3.23 *m*	3.21 *m*	3.2 *m*	3.01 *m*	3.16 *m*
H-2	4.97 *d* (6.2)	5.10 *d* (5)	4.92 *d* (6)	4.95 *d* (7.3)	4.89 *d* (6)
H-4	5.58 *br s*	6.4 *br s*	5.53 *br s*	4.99 *br s*	5 50 *m*
H-5	3.03 *m*	3.03 *m*	2.99 *m*	2.92 *m*	2.88 *m*
H-6	4.84 *d* (6.7)	4.92 *d* (6.7)	4.82 *d* (7)	4.75 *d* (7)	4.77 *d* (6)
$H\text{-}8_{ax}$	3.93 *dd* (2, 9)	4.02 *dd* (2, 9)	3.95 *dd* (9, 2)	3.92 *dd* (2, 9)	3.95 *dd* (2, 9)
$H\text{-}8_{eq}$	4.18 *dd* (5.7, 9)	4.24 *dd* (5, 9)	4.19 *dd* (5, 9)	4.07 *dd* (5, 9)	4.18 *dd* (6, 9)
Ar–H	6.88–7.06	6.92–7.0	6.75–7.05	6.81–6.98	6.6–7 1 *m*
OCH_3	3.90 *s*	3.80 *s*	3.82 *s*	3.83, 3.81, 3.34 *s*	
Aliphatic Ac		1.96 *s*			
Aromatic Ac		2.26 *s*			
$-OCH_2O-$					5.89 *s*

the study of mass spectra of **1**, **2**, **3** and **4**. Fragment ions at *m/z* 163 and *m/z* 166 obtained as a result of 'vertical' and 'horizontal' cleavages respectively of **1** permitted the placement of the two aryl groups at different heterocyclic rings [4]. The expulsion of elements of H_2O from the molecular ion (*m/z* 356) together with the above evidence suggested this lignan to be an isomer of 4-*hydroxysesamin* **6**, leading to its characterisation as 4-*hydroxy*-2,6-*di*(4'-*hydroxy*-3'-*methoxy*)*phenyl*-3,7-*dioxabicyclo*(3.3.0)*octane* **1**. The stereochemical assignment of C-2 aryl as equatorial was evidenced by observing the chemical shift values of C-8 methylene protons (δ3.93, $H\text{-}8_{ax}$; δ4.18, $H\text{-}8_{eq}$) [5, 6]. However, a conclusive di-equatorial disposition of both C-2 and C-6 aryl groups was inferred by transforming **1** into **5** identified as (+)-*pinoresinol*. The orientation of the OH function at C-4 appears to be responsible for inducing magnetic non-equivalence to the two benzylic methines.



	R^1	R^2	R^3	R^4
1	H	Me	H	OH
2	Ac	Me	H	OAc
3	Me	Me	H	OH
4	Me	Me	H	OMe
5	H	Me	H	H
6	$—CH_2—$		OH	H

Inspection of the molecular model suggests this phenomenon to occur only when the OH group is axially oriented. This steric influence is evident by observing the changes in chemical shift values of H-2 and H-6 during a comparative spectral studies between **1** and **2** (Δ, −0.13 and −0.08 ppm respectively) and **1** and **4** (Δ, +0.02 and +0.09 ppm respectively). Further, the ineffectiveness of the coupling as experienced by C-4 proton signal in **1** (δ5.58 *br s*), **2** (δ6.4 *br s*), **3** (δ5.53 *br s*) and **4** (δ4.99 *br s*) suggests it to be *cis* in relation to H-5 and hence equatorial.

4-*Hydroxysesamin* has also been claimed to possess both of its aryl groups as diequatorial [4]. In the light of the above evidence, therefore, and taking into account the multiplicity of C-4 proton signal in 4-hydroxysesamin, its hydroxyl group should be placed equatorial, leading to its representation as **6**. Other compounds isolated from the $CHCl_3$-soluble fraction were identified as *n*-10-nonacosanol, scopoletin, syringic acid and β-sitosterol. The polar fraction of the ethanol residue afforded β-sitosterol-β-D-glucoside which inhibited 43 and 60% response of the spasmogens at 50 and 100 μg/ml respectively.

EXPERIMENTAL

The mps are uncorr. The ^1H NMR spectra were recorded on Perkin–Elmer R-32 (90 MHz) and Varian CFT-20 (80 MHz) spectrometer using TMS as int standard. MS were recorded using a direct inlet system.

Isolation of constituents. Air-dried powdered aerial parts of *L. hypoleuca* Dcne (2.9 kg) were percolated with 95% EtOH (4 × 8 l.) which after removal of the solvent *in vacuo* gave a residue (155 g). The latter was successively fractionated into hexane (2.5 g), $CHCl_3$ (14 g), *n*-BuOH (45 g) and H_2O (72 g) soluble fractions. Column chromatography of a part of the $CHCl_3$-soluble residue (8 g) over silica gel (320 g, 60–120 mesh) yielded *n*-10-nonacosanol (500 mg), β-sitosterol (15 mg), scopoletin (9 mg), syringic acid (10 mg) and **1**.

4-*Hydroxy*-2,6-*di*-(4'-*hydroxy*-3'-*methoxy*)*phenyl*-3,7-*dioxabicyclo*(3.3.0)*octane* (**1**). 50 mg of **1** eluted with a mixture of $CHCl_3$–MeOH (98:2); IR ν_{max}^{KBr} cm^{-1}: 3400–3100 (OH), 3000–2700 (C–H stretching), 1610, 1500, 1435, 1360, 1275, 1238,

1164, 1130, 1113; UV λ_{max}^{MeOH} nm: (log ε), 281.5 (4.19), 237 (4.58); MS *m/z*: 374 $[M]^+$, 356, 327, 222, 205, 204, 196, 193, 192, 191, 178, 175, 166, 163, 161, 153, 151.03651 ($C_8H_7O_3$, 100%), 137, 131, 124, 115, 109, 103, 93, 81, 77, 70, 65, 55.

4-*Acetoxy*-2,6-*di*(4'-*acetoxy*-3'-*methoxy*)*phenyl*-3,7-*dioxabicyclo*(3.3.0)*octane* (**2**). A mixture of **1** (15 mg), Ac_2O (0.5 ml) and pyridine (1.5 ml) was left overnight at room temp. Usual work-up yielded a viscous mass (12 mg); MS *m/z*: 500 $[M]^+$, 458, 416, 398, 369, 356, 328, 205, 191, 175, 163, 151, 137, 131, 97.

4-*Hydroxy*-2,6-*di*-(3',4'-*dimethoxy*)*phenyl*-3,7-*dioxabicyclo*-(3.3.0) *octane* (**3**). Compound **1** (10 mg) in dry MeOH (1 ml) was methylated with CH_2N_2 in ether to yield **3** (8 mg), mp 146–147° (hexane–CH_2Cl_2); MS *m/z*: 402 $[M]^+$, 384, 355, 341, 325, 281, 264, 236, 219, 210, 205, 192, 189, 177, 167, 165, 151, 139, 131, 119, 77, 69.

4-*Methoxy*-2,6-*di*-(3',4'-*dimethoxy*)*phenyl*-3,7-*dioxabicyclo*-(3.3.0)*octane* (**4**). A soln of **1** (10 mg) and MeI (0.2 ml) in dry acetone, 10 ml) was refluxed in presence of K_2CO_3 for 4 hr. The reaction mixture was filtered, the solvent removed and the residue purified by prep TLC to yield **4** (2 mg), mp 123.5° (hexane–CH_2Cl_2); MS *m/z*: 416 $[M]^+$, 383, 354, 218, 206, 191, 176, 166, 164, 151, 84, 71, 69, 57, 55.

2,6-*Di*-(4'-*hydroxy*-3'-*methoxy*)*phenyl*-3,7-*dioxabicyclo*(3.3.0) *octane* (**5**). A soln of **1** (10 mg) and diborane–DMSO complex in THF (1 ml) was heated at 50° for 4 hr. Excess of the reagent was decomposed by adding a satd aq. NH_4Cl soln to the reaction mixture. The THF was removed *in vacuo* and the resulting residue was extracted with $CHCl_3$ (3 × 20 ml). The organic layer was washed with H_2O, dried (Na_2SO_4) and the solvent removed to give a viscous mass which was purified by prep TLC to afford **5** (2.5 mg) identical in all respects with (+)-*pinoresinol*.

REFERENCES

1. Khan, K. A. and Shoeb, A. (1984) *Phytochemistry* **23**, 765.
2. Vishnoi, S. P., Shoeb, A., Kapil, R. S. and Popli, S. P. (1983) *Phytochemistry* **22**, 597.
3. Sharma, P. N., Shoeb, A., Kapil, R. S. and Popli, S. P. (1981) *Phytochemistry* **20**, 335.
4. Anjaneyulu, A. S. R., Rao, A. M., Rao, K., Row, L. R., Pelter, A. and Ward, R. S. (1977) *Tetrahedron* **33**, 133.
5. Birch, A. J., Macdonald, P. L. and Pelter, A. (1967) *J. Chem. Soc.* 1968.
6. Atal, C. K., Dhar, K. L. and Pelter, A. (1967) *J. Chem. Soc.* 2229.

Phytochemistry, Vol. 24, No. 3, pp. 630–632, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

3-*O*-FERULOYL-4-*O*-CAFFEOYLQUINIC ACID FROM COFFEE BEANS

HIDEO IWAHASHI,* HIDEKO MORISHITA,† NORIKO OSAKA* and RYO KIDO*

*Department of Biochemistry, Wakayama Medical College, Wakayama 640; †Faculty of Education, Wakayama University, Wakayama 640, Japan.

(*Received* 9 *August* 1984)

Key Word Index—*Coffea canephora*; Rubiaceae; robusta coffee bean; feruloyl-caffeoyl quinic acid; chlorogenic acids.

Abstract—A new phenolic ester was isolated from unroasted robusta coffee beans (*Coffea canephora*) by HPLC. The isolated compound was identified as an ester of caffeic acid and ferulic acid with quinic acid (3-*O*-feruloyl-4-*O*-caffeoylquinic acid) using ^1H NMR and mass spectroscopy.

INTRODUCTION

Quinic acid esters of hydroxycinnamic acid are of interest in plant physiology and food chemistry because of their ubiquitous occurrence in plants. Considerable amounts of them are contained in unroasted coffee beans [1–4]. The most common compounds are esters of caffeic acid, that is, chlorogenic acid and its derivatives [5]. They are often found together with ferulic acid esters [6].

Because of the structural similarity of chlorogenic acid derivatives, a highly effective separation method is required to resolve all compounds [7]. We have now developed a HPLC method which allows a clear separation of the possible positional isomers [8]. Seven phenolic compounds in unroasted coffee beans have been isolated by this method and the structure of the compounds have been confirmed by mass and ^1H NMR spectroscopy. In the course of separation of the phenolic compounds using reversed-phase HPLC we noticed an additional peak and isolated the compound. The isolated compound was identified as 3-*O*-feruloyl-4-*O*-caffeoylquinic acid by using mass and ^1H NMR spectroscopy and forms the subject of the present report.

RESULTS AND DISCUSSION

Crude green coffee bean extract was applied to a Fine Sil C_{18} semi-preparative column with 10 mM H_3PO_4 and methanol as eluents. By employing a combination of