Phytochemistry, 1973, Vol. 12, pp. 1799 to 1803. Pergamon Press. Printed in England.

NEW LIGNANS FROM LEAVES OF *MACROPIPER EXCELSUM*

GRAEME B. RUSSELL
Applied Biochemistry Division, D.S.I.R., Palmerston North, New Zealand

and

PETER G. FENEMORE
Entomology Division, D.S.I.R., Nelson, New Zealand

(*Received* 13 *November* 1972. *Accepted* 23 *January* 1973)

Key Word Index—*Macropiper excelsum*; Piperaceae; lignans; (+)-diayangambin; (+)-excelsin; (+)-epiexcelsin; (+)-demethoxyexcelsin.

Abstract—Four new lignans, (+)-diayangambin; (+)-excelsin; (+)-epiexcelsin and (+)-demethoxyexcelsin, were isolated from leaves of *Macropiper excelsum* and their structures and configurations have been determined by spectroscopic studies. Diayangambin is only the second naturally occurring diaxially substituted 3,7-dioxabicyclo-[3,3,0]-octane to have been isolated.

INTRODUCTION

Macropiper excelsum (Forst. f.) Miq. is a shrub of the family Piperaceae found near the coast throughout New Zealand. The leaf and bark have been reported as cures for cuts, wounds, stomach pains and toothache[1] while the bitter smoke from slowly burning leaves has been used as an insecticide.[1] The essential oil has yielded a number of compounds, including myristicin[2] which as the predominant product probably accounts for the antiseptic and insecticidal activity.[3]

During a survey of elements of the New Zealand flora for insect control chemicals[4] we became interested in *M. excelsum* because leaf material when incorporated into a milk diet was toxic to house-fly larvae. Chromatography of extracts of the leaves gave an active fraction from which we have isolated, amongst other compounds, four neutral lignans. This report deals with the structures of these lignans.

RESULTS

Macropiper excelsum leaves were extracted and fractionated, as described in the Experimental, to give the hitherto unknown lignans, (+)-diayangambin (I), (+)-excelsin (II), (+)-epiexcelsin (III) and (+)-demethoxyexcelsin (IV).

The NMR spectra (Table 1) indicated these compounds to be 2,6-diaryl-3,7-dioxabicyclo-[3,3,0]-octanes, i.e. lignans of the fused bistetrahydrofuran series; and the assignment of signals due to aromatic protons, methylenedioxy protons and methoxyl protons established the pattern of substitution on the aromatic moieties. In lignans of this class three types of

[1] BROOKER, S. G. and COOPER, R. C. (1962) *New Zealand Medicinal Plants*, p. 30, Unity Press, Auckland, N.Z.
[2] BRIGGS, L. H. (1941) *J. Soc. Chem. Ind. (Lond.)* **60**, 210.
[3] LICHTENSTEIN, E. P. and CASIDA, J. E. (1963) *J. Agr. Food Chem.* **11**, 410.
[4] RUSSELL, G. B., FENEMORE, P. G. and SINGH, P. (1972) *Australian J. Biol. Sci.* **25**, 1025.

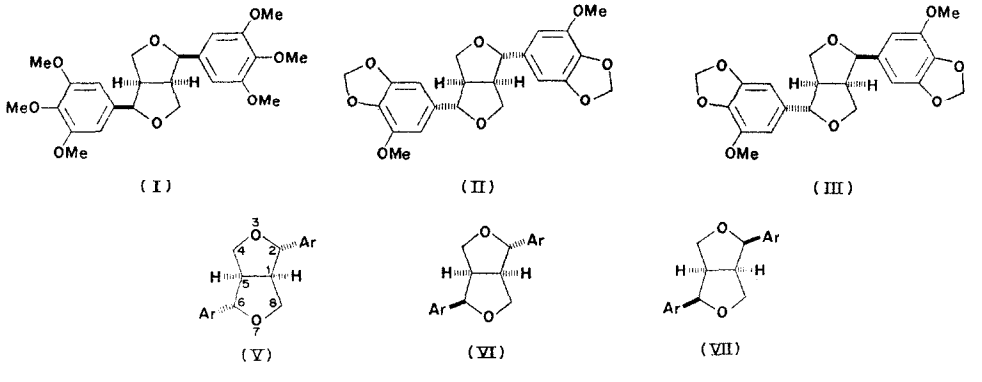
stereoisomers are possible, the isomer with both aryl groups equatorial (V), the isomer with one aryl group equatorial and one axial (VI) and the isomer with both aryl groups axial (VII).

TABLE 1. NMR SPECTRA OF LIGNANS*

Protons	Eudesmin V	Epieudesmin VI	Diaeudesmin VII	Diayangambin I	Excelsin II	Epiexcelsin III	Demethoxyexcelsin IV
1H	3·15*m*	2·9*m*	3·15*m*	3·18*m*	3·0*m*	2·80*m*	3·18*m*
2H	4·75 *d*; *J*,4	4·85 *d*, 5·5	4·90 *d*, 5	4·90 *d*, 5	4·67 *d*, 5	4·76 *d*, 5	4·72 *d*, 5
4αH	4·2–4·4*m*	3·7–3·9*m*	3·65–4·0*m*	3·70–3·90*m*	4·10–4·36*m*	3·67–3·95*m*	4·14–4·40*m*
4βH	3·8–4·0*m*	3·25–3·45*m*	3·3–3·65*m*	3·40–3·70*m*	3·72–3·97*m*	3·22–3·45*m*	3·75–3·97*m*
5H	3·15*m*	3·3*m*	3·15*m*	3·18*m*	3·0*m*	3·10*m*	3·18*m*
6H	4·75 *d*, 4	4·45 *d*, 7	4·90 *d*, 5	4·90 *d*, 5	4·67 *d*, 5	4·30 *d*, 7	4·72 *d*, 5
8αH	4·2–4·4*m*	4·1–4·4*m*	3·65–4*m*	3·70–3·90*m*	4·10–4·36*m*	4·00–4·22*m*	4·14–4·40*m*
8βH	3·8–4·0*m*	3·7–3·9*m*	3·3–3·65*m*	3·40–3·70*m*	3·72–3·97*m*	3·67–3·95*m*	3·75–3·97*m*
OCH_3	3·86, 3·90	3·86, 3·90	3·86, 3·90	3·88, 3·90	3·90	3·90	3·92
O_2CH_2					5·94	5·95	5·94
Aromatic	6·8–7·0*m*	7·1–6·9*m*	6·8–7·0*m*	6·59*m*	6·50*s*	6·55*s*	6·8*m*, 6·54*s*

* All values of δ relative to TMS; all spectra run in $CDCl_3$ at 60 MHz; coupling constant J in Hz.

In a NMR study[5] of (+)-eudesmin (V), (+)-epieudesmin (VI) and (+)-diaeudesmin (VII) it has been shown (Table 1) that the symmetrical diequatorial and diaxial isomers have four aliphatic protons in different environments, rather than eight, while the axial-equatorial isomer has a methine C-1 proton different from that at C-5, and the benzylic proton at C-2 shows as a doublet at a different chemical shift from that due to 6-H. The positions of the C-4, C-8 methylene protons are also characteristic of each of the three isomers. The axial aryl groups in diaeudesmin (VII) are held very close to the axial protons in the opposite rings so these protons resonate upfield (δ3·3–3·7) from the 'normal' position (δ3·8–4·0) by



Ar = 3′, 4′-Dimethoxyphenyl

the anisotropic effect of the aromatic ring. In the case of the diequatorial isomer (V) the two equatorial methylene protons are moved downfield to δ4·2–4·4 due to the deshielding effect of the equatorial aryl groups. In the remaining isomer (VI) one proton resonance moves downfield to δ4·2–4·4 and one moves upfield to δ3·25–3·45 corresponding to one equatorial and one axial aryl group respectively. By comparing NMR spectra with those of these three compounds (Table 1) the complete stereochemistry of the new lignans could be established.

The optical rotations of the new lignans paralleled the rotations of compounds of known

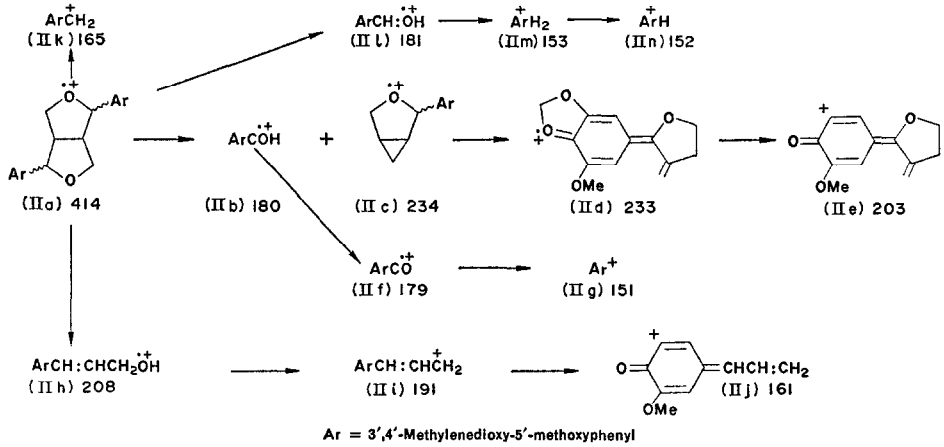
[5] BIRCH, A. J., MACDONALD, P. L. and PELTER, A. (1967) *J. Chem. Soc.* C, 1968.

absolute configuration and this confirmed the assignment of the stereochemistry. In the eudesmin series,[6] (+)-eudesmin has $[\alpha]_D$ +64°, (+)-epieudesmin has $[\alpha]_D$ +119° and (+)-diaeudesmin has $[\alpha]_D$ +316°; the sesamin series gives similar figures.[7] With the new compounds, (+)-excelsin has $[\alpha]_D$ +44°, (+)-demethoxyexcelsin has $[\alpha]_D$ +54°, (+)-epiexcelsin has $[\alpha]_D$ +131°, and (+)-diayangambin has $[\alpha]_D$ +289°.

The IR and UV spectra served to confirm the presence or absence of the methylenedioxy groups while the positions of IR bands were consistent with the stereochemistry proposed.[6]

(+)-*Diayangambin* (I)

The molecular formula $C_{24}H_{30}O_8$ was assigned from elemental analysis and the mass measurement of the molecular ion in the high resolution MS. The MS showed one series of fragmentations characteristic of that described for lirioresinol-*B* dimethyl ether.[8] This latter compound is the diequatorial stereoisomer of I and is, in fact, identical to yangambin isolated from *Piper guineese*.[9] That diayangambin is a 3,7-dioxabicyclo-[3,3,0]-octane with two 3′,4′,5′-trimethoxyphenyl groups is supported by the NMR spectrum (Table 1) which shows the presence of six methoxyl groups at δ3·90 and 3·88 while the four *meta* aromatic protons occur as a singlet at δ6·59. All other features of the NMR spectrum are consistent with those of (+)-diaeudesmin and I must be the diaxial isomer. This is confirmed by the optical rotation and I is the second reported occurrence of a natural diaxially substituted lignan of the fused bistetrahydrofuran series.



SCHEME 1. FRAGMENTATION PATTERN OF EXCELSIN.

(+)-*Excelsin* (II)

The molecular formula $C_{22}H_{22}O_8$ of this new compound was assigned from the elemental analysis and the MS. The main fragmentation pattern[10] shown in Scheme 1, is consistent with the 3,7-dioxabicyclo-[3,3,0]-octane skeleton, the C2 and C6 positions of which have 3′,4′-methylenedioxy-5′-methoxyphenyl groups. The formation of the ions outlined is

[6] ATAL, C. K., DHAR, K. L. and PELTER, A. (1967) *J. Chem. Soc.* **C**, 2228.
[7] FREUDENBERG, K. and SIDHU, G. S. (1960) *Tetrahedron Letters* 3.
[8] KAKISAWA, H., CHEN, Y. P. and HSU, H. Y. (1972) *Phytochemistry* **11**, 2289.
[9] HANSEL, R., LEUCKERT, C. H. and SCHULZ, G. (1966) *Z. Naturforsch*, **21b**, 530.
[10] PELTER, A. (1967) *J. Chem. Soc.* **C**, 1376.

supported by mass measurement and the presence of meta stable peaks. The scheme of Pelter[10] for the fragmentation of eudesmin has been extended to account for ions IIe and IIj which arise by loss of formaldehyde from the ions IId and IIi respectively. Another species at *m/e* 178 probably originates from IIh by a similar loss of formaldehyde.

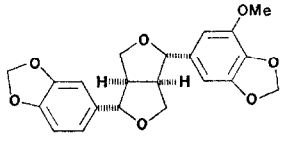
The NMR spectrum confirmed that (+)-excelsin had two methoxy groups (δ3·90), four methylenedioxy protons (δ5·94) and four aromatic protons (δ6·50). The aromatic protons occurred as a singlet and must be the 2′,6′-aryl protons; the methoxyl groups are substituted on the 5′ positions. The chemical shifts and coupling of the aliphatic protons (Table 1) were consistent with those of (+)-eudesmin and the spectrum had no signals between δ3·2 and 3·7; (+)-excelsin is therefore the diequatorial stereoisomer and has the appropriate optical rotation.

(+)-*Epiexcelsin* (III)

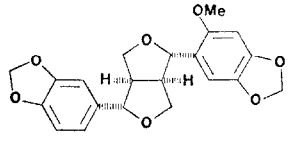
Apart from intensity differences this material gave an identical MS to excelsin with the fragmentation pattern of Scheme 1. Its optical rotation and NMR spectrum suggested that it was the axial-equatorial stereoisomer of (+)-excelsin with signals due to the benzylic, methine and methylene protons (Table 1) identical to those for (+)-epieudesmin. The methoxyl, methylenedioxy and aromatic protons occurred at chemical shifts consistent with those of excelsin. The optical rotation is similar to that of (+)-epieudesmin and occurs approximately half way between the rotations of (+)-diayangambin and (+)-excelsin.

(+)-*Demethoxyexcelsin* (IV)

The fourth compound isolated had molecular formula $C_{21}H_{20}O_7$ from elemental analysis and the MS. Its MS pattern was more complicated than those of I, II and III and revealed two series of fragmentations. One series is identical to the spectrum of excelsin in that all the ions IIb–IIn in Scheme 1 could be recognized. The species of the other series have 30 mμ smaller mass number and the pattern is similar to that in Scheme 1 and identical to that of sesamin.[10] IV therefore has the 3,7-dioxabicyclo-[3,3,0]-octane skeleton, the C-2 and C-6 positions of which have 3′4′-methylenedioxy-5′-methoxyphenyl and 3″,4″-methylenedioxyphenyl groups attached respectively.



(IV)



(VIII)

The NMR spectrum showed the presence of two methylenedioxy groups at δ5·94 and one methoxyl group at δ3·92. Aromatic protons appeared at two positions, a two proton singlet at δ6·54 which was consistent with the corresponding signal of (+)-excelsin and indicated the methoxyl group was on the 5′ position, and a three proton multiplet whose shape and chemical shift is identical to a corresponding signal in sesamin.[11] The other features of the NMR indicate that IV has the same stereochemistry as (+)-eudesmin and is the diequatorial isomer.

[11] BECKER, E. D. and BEROZA, M. (1962) *Tetrahedron Letters* 157.

A similar compound, sesangolin,[12] has been isolated and identified as VIII; i.e. demethoxyexcelsin with the methoxyl in the 6′ position rather than 5′. Although the melting points of these materials are the same, the NMR spectra in the aromatic region are different, with sesangolin having two one-proton singlets at δ6·43 and 6·84. The preparation of a dinitro derivative confirmed the difference of the two compounds.

Macropiper excelsum has furnished an interesting series of lignans and is the first plant to yield a representative of each type of stereoisomer possible with 2,3-diaryl-3,7-dioxabicyclo-[3,3,0]-octanes. Some of these materials appear to have slight insect toxicity and this may be correlated to the synergistic and juvenile hormonal effect of the related compound, sesamin.[13] Other components of the benzene fraction, however, account for greater activity.

EXPERIMENTAL

Isolation of the lignans. The dried milled foliage (2·3 kg) of *Macropiper excelsum* (voucher specimen CHR234165) was extracted in a Soxhlet apparatus with EtAc for several hours. The solvent was removed under reduced pressure and the residue was dissolved in 85% MeOH–H_2O and partitioned between this solvent and petrol. (b.p. 40–60°). The aqueous layer was removed and extracted 2× more with petrol. The petrol. layers were each extracted in turn with a second portion of MeOH–H_2O. The combined aqueous layers were concentrated to dryness under reduced pressure, the residue (52 g) dissolved in C_6H_6 and the solution eluted through a column of neutral alumina (Woelm, activity IV, 350 g) with C_6H_6 (5 l.).

The eluate was concentrated to dryness and the residue (23 g) was partitioned between petrol., diethyl ether, MeOH and H_2O (8:5:3:1). The bottom, methanolic layer was concentrated to dryness (10 g) and the semicrystalline solid recrystallized from MeOH to give diayangambin. The top Et_2O layer was concentrated to dryness (12·7 g) and the product chromatographed on silicic acid (Mallinckrodt, 150 g, 5% H_2O). Elution with petrol. spirit (40–60°), EtAc (4:1) gave a series of fractions which were combined according to their component analysis on TLC and subjected to further chromatography on silicic acid in the same solvent. Three crystalline compounds were obtained and were recrystallized from MeOH.

(+)-*Diayangambin.* (5·4 g) M.p. 151–2°. $[\alpha]_D^{18}$ +289° ($CHCl_3$) (*Anal.* Found: C, 64·41; H, 6·62% Calc. for $C_{24}H_{30}O_8$: C, 64·50; H, 6·81%). IR ($CHCl_3$). 1600, 1510, 1470, 1420, 1370, 1240, 1130, 1090, 1010, 930 cm^{-1}. UV (EtOH): 270, 230 nm; ε, 1680, 17000.

(+)-*Excelsin.* (184 mg) M.p. 122–3°. $[\alpha]_D^{18}$ +44° ($CHCl_3$) *Anal.* Found: C, 63·72; H, 5·42% Calc for $C_{22}H_{22}O_8$: C, 63·91; H, 5·32%). IR ($CHCl_3$). 1640, 1510, 1450, 1430, 1310, 1200, 1140, 1090, 1045, 930, 830 cm^{-1}. UV (EtOH): 275 242 nm; ε, 2900, 10500.

(+)-*Epiexcelsin.* (168 mg) M.p. 164·5°–5·5°. $[\alpha]_D^{18}$ +131° ($CHCl_3$) (*Anal.* Found: C, 63·57; H, 5·17% Calc for $C_{22}H_{22}O_8$: C, 63·91; H, 5·32%). IR ($CHCl_3$). 1640, 1510, 1450, 1430, 1360, 1310, 1195, 1140, 1090, 1075, 1036, 970, 935, 830 cm^{-1}. UV (EtOH): 275, 242 nm; ε, 2800, 11 000.

(+)-*Demethoxyexcelsin.* (123 mg) M.p. 101–2°. $[\alpha]_D^{18}$ +54·2° ($CHCl_3$) (*Anal.* Found: C, 65·42; H, 5·28% Calc for $C_{21}H_{20}O_7$: C, 65·6; H, 5·21%). IR ($CHCl_3$). 1640, 1500, 1440, 1250, 1140, 1095, 1045, 940, 830, 815 cm^{-1}. UV (EtOH): 285, 237 nm; ε, 5250, 9700. M.p. of the dinitro derivative[12] 198–9°.

Acknowledgements—The authors are indebted to Professor R. Hodges, Massey University, for the high resolution MS and to Mrs. M. Shortall for technical assistance.

[12] JONES, W. A., BEROZA, M. and BECKER, E. D. (1962) *J. Org. Chem.* **27**, 3232.
[13] BOWERS, W. S. (1968) *Science* **161**, 895.