Phytochemistry, Vol. 27, No. 7, pp. 2309–2312, 1988.
Printed in Great Britain.

0031 9422/88 $3.00 + 0.00

METABOLITES FROM *CHLOROPHORA EXCELSA*: POSSIBLE INTERMEDIATES IN THE BIOGENESIS OF A PENTASUBSTITUTED STILBENE

ELFRANCO MALAN*, EWALD SWINNY, DANEEL FERREIRA and ALIDA J. HALL†

Department of Chemistry, University of Durban-Westville P/BAG X54001, Durban, 4000 South Africa; †Department of Chemistry; University of the Orange Free State, P. O. BOX 339, Bloemfontein, 9300 South Africa

(*Revised received* 25 *November* 1987)

Key Word Index—*Chlorophora excelsa*; Moraceae; 2,4-dihydroxybenzaldehyde; 3,5-dihydroxybenzaldehyde; 3,5-dihydroxy-4-geranylbenzaldehyde; 3′,4,5′-trihydroxy-4′-geranylstilbene; 2,3′,4,5′-tetrahydroxy-4′-geranylstilbene; 2,3′,4,5′-tetrahydroxystilbene; 3,4′-7-tri-*O*-methylquercitin; 2′-methoxy-3,4′,7-tri-*O*-methylquercitin.

Abstract—A series comprising substituted hydroxyl-geranylstilbenes, substituted benzenoid compounds and quercitin-type flavones were isolated and characterized from the acetone extract of the heartwood of *Chlorophora excelsa*.

INTRODUCTION

Chlorophora excelsa is a large tree reaching 50 m in height and occurring in low altitude evergreen forests in central and eastern tropical reagions of Africa [1]. Its wood is an attractive brown and in great demand as timber for building and for heavy duty furniture. The heartwood of *C. excelsa* (Iroko) was previously investigated by King *et al.* [2, 3] who discovered and elucidated the structure of 2,3′,4,5′-tetrahydroxy-4′-geranylstilbene (chlorophorin).

The re-investigation of Iroko for minor metabolites has proved to be justified and an interesting series of potential biogenetic precursors of 2,3′,4,5′-tetrahydroxy-4′-geranylstilbene [3] were isolated. Several tetra- and pentahydroxystilbenes and their derivatives with different substitution patterns were isolated before from *Vouacapoua macropetala* [4], *Eucalyptus* species [5], spruce bark [6], *Schotia brachypetala* [7], *Rheum rhaponticum* L. [8] and *Combretum caffrum* [9].

RESULTS AND DISCUSSION

The acetone extract of the heartwood of *C. excelsa* afforded eight compounds comprising two simple dihydroxybenzaldehydes (**1a** and **1b**), 3,5-dihydroxy-4-geranylbenzaldehyde (**1c**), 3,4′,7-tri-*O*-methylquercitin [10], 2′-methoxy-3,4′,7-tri-*O*-methylquercitin [10], 3′,4,5′-trihydroxy-4′-geranylstilbene (**2a**), 2,3′,4,5′-tetrahydroxy-4′-geranylstilbene (**2b**, chlorophorin) and 2,3′,4,5′-tetrahydroxystilbene (**2c**). The two dihydroxybenzaldehydes (**1a** and **1b**) were isolated and identified with authentic reference compounds commercially available, the 3,5-dihydroxybenzaldehyde **1b** was present in a much lower quantity than the 2,4-isomer **1a**.

The 3,5-dihydroxy-4-geranylbenzaldehyde (**1c**) showed an expected simple NMR pattern (acetone-d_6) in the benzenoid range with a singlet at δ6.93 (2H) accounting for the two equivalent protons at the 2- and 6-positions; a broadened singlet, integrating for two protons at δ8.76 representing the 3- and 5-hydroxyls. A singlet at δ9.75 (1H) is typical of a deshielded aldehyde proton. On treatment of **1c** with methyl sulphate/potassium carbonate the low field singlet remained and the aldehyde carbonyl was confirmed by the IR spectrum of the 3,5-*O*-dimethyl derivative of **1c**. With homodecoupling experiments it was possible to assign the chemical shifts for the protons of the 4-geranyl group (Table 1), which proved to be the same for chlorophorin **2b**. High resolution mass spectrometry of **1c** confirmed the molecular mass to be 274 and the loss of 123 mass units to yield the base peak at *m/z* 151 also confirmed the presence of a geranyl group.

The 3,5-dihydroxy-4-geranylbenzaldehyde (**1c**) was very sensitive to the atmosphere and heat. Once it was purified by preparative TLC it had to be kept under constant vacuum otherwise it turned into a brown residue. It is noteworthy that when the aldehyde **1c** was in a mixture with 2,4-dihydroxyaldehyde (**1a**) in an approximate ratio of 1:2, it was not so sensitive to factors mentioned earlier, which could point towards a type of hydrogen bonding protection.

Starting with 3,5-dihydroxybenzyl alcohol (0.015 mol) in ethanol medium adjusted to pH 1 [11], geraniol (0.020 mol) was added carefully while the reaction mixture was stirred at 60° for 2 hr. An attempt to oxidize the resulting 3,5-dihydroxy-4-geranylbenzyl alcohol to the aldehyde **1c** was not successful. Although various procedures were tried, it always resulted in a dark brown residue. The approach was changed and the isolated aldehyde **1c** was treated with lithium aluminium hydride/ether to give the required 3,5-dihydroxy-4-geranylbenzyl alcohol (62% yield), which confirmed the structure of **1c**.

The ^{1}H NMR (acetone-d_6) of 3′,4,5′-trihydroxy-4′-geranylstilbene (**2a**) showed a AA′ (δ7.37 *m*; 2H) BB′ (δ6.81 *m*; 2H) system for the one benzene ring and a singlet (2H) at δ6.58 accounting for the magnetically identical protons of the other benzene ring. The two

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR data of Iroko metabolites (300 MHz, TMS as int. stand.)

Compound	Aromatic protons 2	3	Aldehyde Proton	5	6	2′	6′	OMe
†**1c**	6.93 *s*		9.75 *s*		6.93 *s*			
***1c** Me ether	7.24 *s*		10.08 *s*		7.24 *s*			4.10(6H, *s*)
†**3a**	6.40 *s*		Benzylic 4.80 *s*		6.40 *s*			
†**2a**	7.37 *d*	6.81 *d*		6.81 *d*	7.37 *d*	6.56 *s*	6.56 *s*	
***2a** Me ether	7.38 *d*	6.83 *d*		6.83 *d*	7.38 *d*	6.61 *s*	6.61 *s*	3.81–3.84 9H, *d*
†**2b**		6.41 *d*		6.35 *m*	7.37 *d*	6.56 *s*	6.56 *s*	

*$CDCl_3$.
†Acetone-d_6.

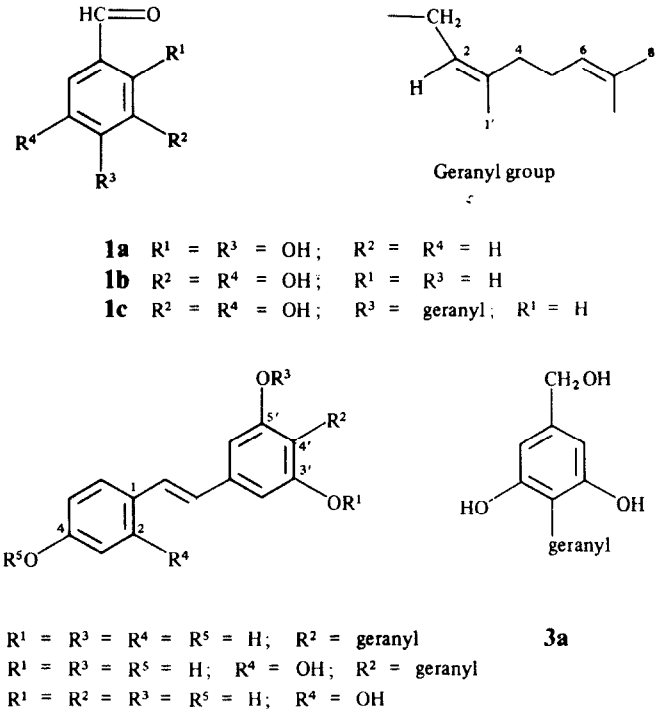


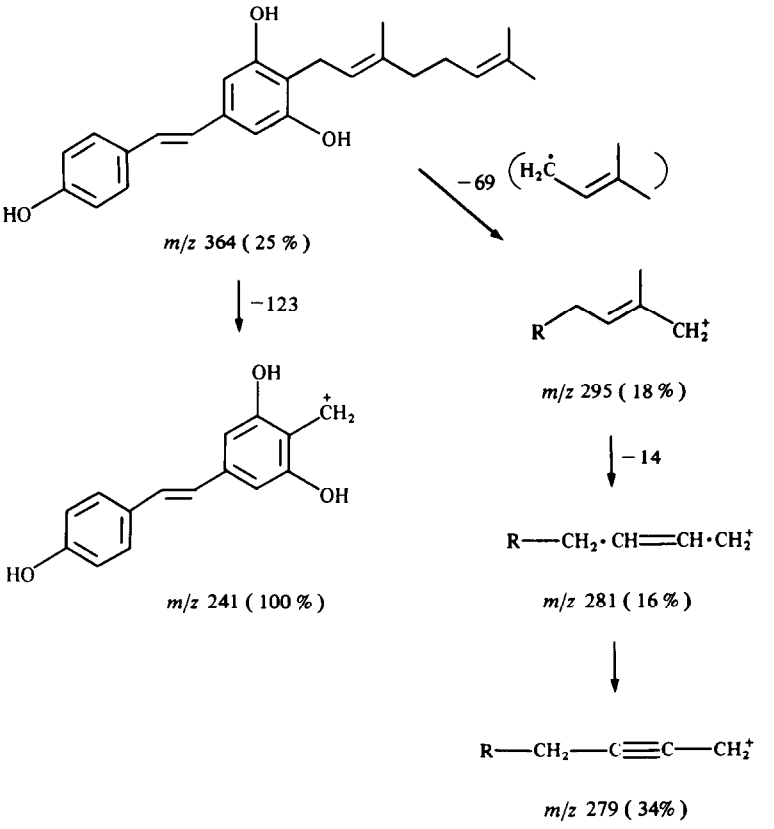
Fig. 1.

olefinic protons with a coupling constant $J = 16.5$ Hz for *trans* protons, appeared as two doublets at δ6.81 and 6.91. The 4′-geranyl group of **2a** showed the same NMR pattern as for compound **1c**.

The mass spectrum of **2a** (Fig. 2) gave a molecular ion at *m/z* 364 (25%) and the fragments *m/z* 295 (18%), 281 (16%), 279 (34%) together with the base peak at *m/z* 241 (100%) confirmed the 4′-geranyl group. The A and B aromatic rings were confirmed by fragments *m/z* 107 (22.4%) and 123 (14%) respectively.

By using the modified Wittig reaction [12], 3,5-dimethoxybenzylbromide was reacted with triethyl phosphite and the resulting phosphonate reacted with 4-methoxybenzaldehyde in dimethylformamide to give 3′,4,5′-trimethoxystilbene (90.4%) in good yield, the stilbene was subsequently demethylated by pyridine hydro-

	Geranyl group protons						
Olefinic	1	2	4	5	6	8	1′
	3.42 *d*	5.29 *m*	1.94 *m*	2.02 *m*	5.06 *m*	1.59 *d* 1.54 *d*	1.77 *d*
	3.58 *d*	5.33 *m*	2.13 *m*	2.22 *m*	5.23 *m*	1.82 *d* 1.75 *d*	1.95 *d*
	3.29 *d*	5.09 *m*	1.95 *m*	2.04 *m*	5.06 *m*	1.61 *d* 1.55 *d*	1.74 *d*
6.91 *d* 6.81 *d*	3.35 *d*	5.31 *m*	1.94 *m*	2.02 *m*	5.07 *m*	1.61 *d* 1.55 *d*	1.77 *d*
6.95 *d* 6.86 *d*	3.27 *d*	5.12 *m*	1.88 *m*	1.97 *m*	5.00 *m*	1.58 *d* 1.50 *d*	1.69 *d*
7.25 *d* 6.80 *d*	3.34 *d*	5.31 *m*	1.94 *m*	2.02 *m*	5.07 *m*	1.61 *d* 1.55 *d*	1,77 *d*



R = 3′,4,5′- trihydroxystilbene group

Fig. 2.

chloride [12] to give the corresponding trihydroxy compound (45%). The 3′,4,5′-trihydroxystilbene was reacted with geraniol under the same conditions as applied for the synthesis of **1c** to yield 3′,4,5′-trihydroxy-4′-geranylstilbene (**2a**, 8%).

EXPERIMENTAL

Mps: uncorr. IR: $CHCl_3$; ^1H NMR: 300 MHz.

Plant material. The heartwood material was kindly supplied by Mr J. Vorster of the Department of Indian Education In-

spectorate in Durban. A specimen of the heartwood was kindly confirmed by Dr B. J. Ter Welle University of Utrecht, Institute for Systematic Botany, Utrecht, Netherlands.

Extraction and isolation. Air-dried milled heartwood was defatted with hexane followed by extraction with Me_2CO and the extract evapd to dryness under red. pressure. The dry Me_2CO extract (24 g) was treated with $CHCl_3$ to concentrate the compounds with a high R_f value when analysed on TLC, (C_6H_6–Me_2CO, 9:1).

The dry $CHCl_3$ extract (4.4 g) was chromatographed over Kieselgel 60 (Merck 7734) and the first 20 fractions eluted with a C_6H_6–Me_2CO (9:1) mixture (40 ml each) and the next 20 fractions with a C_6H_6–Me_2CO (2:1) mixture. Fractions 12–20 contained compounds **1a**, **1b**, **1c**, **2a** and were separated by prep. TLC (C_6H_6–Me_2CO, 9:1). The *O*-methyl derivatives were prepared by the $(Me)_2SO_4/K_2CO_3$ method.

3,5-*Dihydroxy*-4-*geranyl benzaldehyde* (**1c**). Non-crystalline, 35 mg (R_f 0.26), mp 80–81°. Found *m/z* 274.1547 ($C_{17}H_{22}O_3$ requires 274.1568).

3,5-*Dimethoxy*-4-*geranyl benzaldehyde.* Non-crystalline, gummy compound, 22 mg (from 20 mg **1c**). IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1700. (Found: C, 75.34; H, 8.75. $C_{19}H_{26}O_3$ requires: C, 75.46; H, 8.67). Accurate mass found *m/z* 302.1879, requires 302.1880.

3,4,5′-*Trihydroxy*-4-*geranylstilbene* (**2a**). Non-crystalline (R_f 0.23), mp 138–140°, 152 mg. (Found: C, 79.113; H, 7.673. $C_{24}H_{28}O_3$ requires: C, 79.077; H, 7.748. Accurate mass found *m/z* 364.2045 (requires 364.2036); *m/z* 241.0866 (requires 241.0863, see Fig. 2).

3′4,5′-*Trimethoxy*-4′-*geranylstilbene.* Non-crystalline, 47 mg (from 45 mg **2a**), mp 74–75°. Found *m/z* 406.2504. ($C_{27}H_{34}O_3$ requires 406.2505).

2,3′,4,5′-*Tetrahydroxy*-4′-*geranylstilbene* (**2b**). Non-crystalline, light brown in colour, mp 154–156° (Lit. [2] 157–159°). $[M]^+$ at *m/z* 380 (22%). Tetra-*O*-methyl ether of **2b**, mp 73–74°, $[M]^+$ at *m/z* 436 (31%).

2,3′,4,5′-*Tetrahydroxystilbene* (**2c**). Non-crystalline, light brown in colour, mp 99–103° [M]; at *m/z* 244 (100%). ^1H NMR, acetone-d_6: δ6.42 (1H, *d*, H-3), 6.34 (1H, *m*, H-5), 7.36 (1H, *d*, H-6). 6.50 (2H, *d*, H-2′ and H-6′), 6.21 (1H, *t*, H-4′), 7.30 (1H, *d*, olefinic), 6.82 (1H, *d*, olefinic).

3′,4,5′-*Trihydroxystilbene.* Light brown needles, mp 260° (Lit. [13] 261°). $[M]^+$ at *m/z* 228 (100%). ^1H NMR, acetone-d_6: δ7.40 (2H, *d*, H-2 and H-6), 6.82 (2H, *d*, H-3 and H-5), 6.56 (2H, *d*, H-2′ and H-6′), 6.27 (1H, *t*, H-4′), 7.05 (1H, *d*, olefinic), 7.82 (1H, *d*, olefinic).

3′,4,5′-*Tri*-O-*methylstilbene.* Light yellow needles, mp 55–57° (Lit. [14] 56–57°). $[M]^+$ at *m/z* 270 (100%). ^1H NMR, $CDCl_3$: δ7.44 (2H, *d*, H-2 and H-6), 6.88 (2H, *d*, H-3 and H-5), 6.64 (2H, *d*, H-2′ and H-6′), 6.37 (1H, *t*, H-4′), 7.03 (1H, *d*, olefinic), 6.89 (1H, *d*, olefinic), 3.84 (9H, *s*, -OMe).

Acknowledgements—We are pleased to thank S. A. Cyanamid and Essential Sterolin Products for their very necessary financial assistance, also for the support obtained from the Research Committee of the University of Durban-Westville. We thank Professor E. V. Brandt and Mr J. Burger of the University of the Orange Free State for the NMR spectra.

REFERENCES

1. Palgrave, K. C. (1977) in *Trees of Southern Africa*, p. 101. Struik, Cape Town.
2. King, F. E. and Grundon, M. F. (1949) *J. Chem. Soc.* 3348.
3. King, F. E. and Grundon, M. F. (1950) *J. Chem. Soc.* 3547.
4. King, F. E., King, T. J., Godson, D. H. and Manning, L. C. (1956) *J. Chem. Soc.* 4477.
5. Hathway, D. E. (1962) *Biochem. J.* **83**, 80.
6. Cunningham, J., Haslam, E. and Haworth, D. (1963) *J. Chem. Soc.* 2875.
7. Drewes, S. E. and Fletcher, I. P. (1974) *J. Chem. Soc. Perkin Trans I*, 961.
8. Kashiwada, Y., Nonaka, G. and Nishioka, I. (1984) *Chem. Pharm. Bull.* **32**, 3501.
9. Pettit, G. R., Singh, S. B., Niven, M. L., Hamel, E. and Schmidt, J. M. (1987) *J. Nat. Prod.* **50**, 119.
10. Malan, E. and Roux, D. G. (1979) *J. Chem. Soc. Perkin Trans I*, 2696.
11. Young, D. A., Ferreira, D. and Roux, D. G. (1985) *J. Chem. Soc. Perkin Trans I*, 2529.
12. Bachelor, F. W., Loman, A. A. and Snowdon, L. R. (1970) *Can. J. Chem.* **48**, 1554.
13. *Dictionary of Organic Compounds* 4th Edn, Vol. 5, p. 3148. Eyre and Spottiswoode, London.