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STRUCTURE AND STEREOCHEMISTRY OF LIMONOIDS OF *CABRALEA EICHLERIANA**

M. MADHUSUDANA RAO†‡, HAIM MESHULAM‡
RAYMOND ZELNIK§ and DAVID LAVIE‡

‡Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel
and
§Serviço de Química, Orgânica, Institute Butantan, São Paulo, Brasil

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Key Word Index—*Cabralea eichleriana*; Meliaceae; limonoids; photolimonoids; dammarane triterpenes; structure determination.

Abstract—From *Cabralea eichleriana* seeds, the following compounds have been isolated: cabraleone, cabraleadiol, fissinolide, 3-deacetylfissinolide, gedunin, 7-deacetoxy-7-oxogedunin, 7-deacetoxy-7-hydroxygedunin, methyl angolensate, and three new compounds, 7-deacetoxy-7-hydroxyphotogedunin, cabralin, and isocabralin. Cabralin has also been obtained by photolysis of fissinolide.

INTRODUCTION

Since the publication of the constitution of limonin, a large number of related compounds have been isolated and characterized in quick succession from 2 closely related families, Rutaceae and Meliaceae [2, 3]. Structural variations exhibited by limonoids of Meliaceae provided ample circumstantial evidence for the biogenetic pathways involved in their formation through oxidative modification of the tetracyclic apoeuphane skeleton. However, only in Rutaceae, limonoids with cleaved ring A modifications are encountered, with methyl ivorensate [4] being probably the only compound of this type isolated from a species of Meliaceae.

The apoeuphane skeleton has been assumed to have formed from squalene through the intermediate stages of dammarane and euphane. Simultaneous occurrence of melianes [5] (having a euphane skeleton) together with limonoids supports this assumption. Aglaiol [6], the first compound with a dammarane skeleton found in Meliaceae, was however, not known to occur along with limonoids. In this context, the isolation of a number of triterpenes with a dammarane skeleton, having ring A intact or cleaved, along with a limonoid (fissinolide) reported earlier [1], from the wood of *Cabralea eichleriana*, is significant.

RESULTS AND DISCUSSION

In the light of these results we have reinvestigated the seeds of *C. eichleriana*. From the light petroleum extract we have now isolated cabraleone (**1**) and cabraleadiol (**2**) previously obtained from the wood [1], whereas the $CHCl_3$ extract afforded in addition to fissinolide (**3**) and the corresponding 3-deacetyl compound **4** already reported [7], the following compounds: gedunin (**5**), 7-deacetoxy-7-oxo-gedunin (**6**), 7-deacetoxy-7-hydroxygedunin (**7**), methyl angolensate (**8**) and 3 new compounds characterised as 7-deacetoxy-7-hydroxyphotogedunin (**9**), cabralin (**12**) and isocabralin (**14**). Compounds **5**–**8** have been identified by their spectral characteristics [8] and confirmed by direct comparison with authentic samples. In this paper evidence leading to the determination of structures **9**, **12** and **14** is presented.

7-Deacetoxy-7-hydroxyphotogedunin (**9**) has a molecular formula $C_{26}H_{32}O_8$; it is insoluble in

* Part 2 in a series on *Cabralea eichleriana* extractives; for Part 1, see ref. [1].

† On leave of absence from Kurukshetra University, Kurukshetra (Haryana), India.

Table 1. NMR Spectral data (δ values)

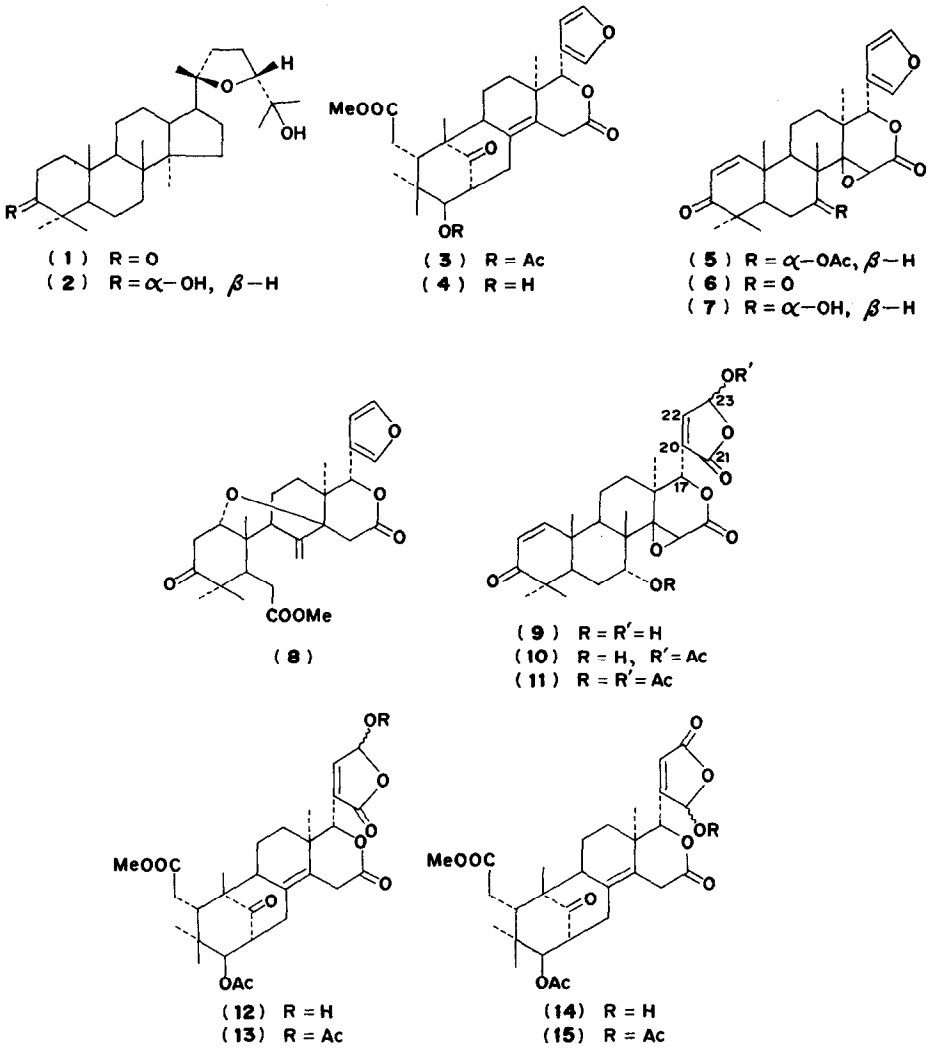
Compound	C-CH_3	1-H	2-H	3-H	7-H	15-H	17-H	21-H	22-H	23-H	$COOCH_3$	$OCOCH_3$
7-Deacetoxy-7-hydroxy-gedunin (**7**)*	0·98(3), 1·10 1·15	7·26 (*d*, J = 10 Hz)	5·73 (*d*, J = 10 Hz)			3·93 (*s*)	5·54	7·67	6·49	7·67		
7-Deacetoxy-7-hydroxy-photogedunin (**9**)*	0·98(3), 1·12 1·17	7·27 (*d*, J = 10 Hz)	5·75 (*d*, J = 10 Hz)			3·95 (*s*)	5·32		7·48	6·26		
7-Deacetoxy-7-hydroxy-23-acetyl-photogedunin (**10**)	1·09(2), 1·14 1·21, 1·23	7·13 (*d*, J = 10 Hz)	5·85 (*d*, J = 10 Hz)		3·54 (*m*)	3·92 (*s*)	5·56		7·35	6·92		2·18
Photogedunin acetate (**11**)	1·10(2), 1·19 1·24, 1·27	7·13 (*d*, J = 10 Hz)	5·86 (*d*, J = 10 Hz)		4·55 (*m*)	3·53 (*s*)	5·57		7·36	6·92		2·10 2·18
Fissinolide (**3**)	0·73, 0·81 1·07, 1·16			5·00 (*d*, J = 10 Hz)			5·72 (*s*)	7·44 (or 7·58)	6·50	7·58 (or 7·44)	3·72	2·18
Cabralin (**12**)	0·74, 0·79 1·05, 1·17			5·00 (*d*, J = 10 Hz)			5·78		7·38	6·27	3·67	2·17
Cabralin acetate (**13**)	0·73, 0·78 1·07, 1·15			5·00 (*d*, J = 10 Hz)			5·83		7·38	6·94	3·67	2·17 (2)
Isocabralin acetate (**15**)	0·70, 0·80 1·12, 1·16			4·98 (*d*, J = 10 Hz)			5·45	6·91	6·40		3·67	2·15 2·18

* Recorded in DMSO.

$CHCl_3$, its NMR spectrum in DMSO is similar to that of 7-deacetoxy-7-hydroxygedunin (**7**) (Table 1), except that the furan proton signals are replaced by two one-proton signals at δ 6·26 and 7·48 together with a broadening of the one due to the 17-H. Upon acetylation, **9** gave a mixture of mono- and diacetates, **10** and **11** respectively, which could be separated by PLC. In the monoacetate the 7-OH is still free as is shown by the appearance of the 15-H signal at δ 3·92, which is at δ 3·53 in the diacetate (**11**). The spectrum of **11** is identical with that of photogedunin acetate [9], thus establishing the structure of the hydroxy compound as **9**. This was further supported by its MS fragmentation. Of the two compounds cabralin (**12**) and isocabralin (**14**), only the former could be obtained in pure state; molecular formula $C_{29}H_{36}O_{10}$. Isocabralin (**14**) was purified by PLC of the mixture containing both compounds after acetylation, and was obtained as the acetate.

The NMR spectrum of cabralin is similar to that of fissinolide [10] (**3**), except for the furan protons which are missing. Instead, it has two one-proton signals at δ 6·27 and 7·38, the former being shifted to 6·94 upon acetylation. These data were suggestive of a side chain structure as encountered in 7-deacetoxy-7-hydroxyphotogedunin (**9**). Isocabralin acetate (**15**) has a molecular formula as cabralin acetate (**13**) $C_{31}H_{38}O_{11}$, as well as a similar fragmentation in the mass spectrum. The NMR spectra of both compounds are also very much alike but instead of the one signal at δ 2·17 for the two acetate methyl groups in **13**, **15** shows two separate signals at δ 2·15 and 2·18, as well as different chemical shifts for the 17-H and the side chain protons. The signal at δ 6·91 is attributed to the 21-H, a proton not present in **13**. The second proton, the vinylic 22-H, which appears at a higher field (δ 6·40) compared with that of cabralin acetate (**13**) (δ 7·38), was assigned the α position to the lactone carbonyl function against its β location in **13**, leading thereby to the two side chain structures as in **12** and **14** for cabralin and isocabralin respectively.

In order to confirm the structure of **12**, a solution of fissinolide (**3**) in C_6H_6 was irradiated with a Pyrex filtered UV light in a stream of O_2 until all the starting material was used up. The reaction product upon chromatography (Si gel) yielded cabralin (**12**) in 30% yield. No isocabralin (**14**) could be detected in the reaction mixture. Interestingly, photolysis of 7-deacetoxy-7-oxogedunin (**6**) under similar conditions [11], as well as gedunin (**5**) in presence of sunlight, were also reported to have yielded only one product each, having the same side chain as in cabralin. Viewed on a Dreiding model, the isomeric side chain structure (as in **14**) shows a strong steric interaction between the 21-OH and the C-13αMe which may



explain the absence of such a product in the reaction mixture.

It could, therefore, be concluded that the 2 compounds cabralin and isocabralin, are genuine natural products, the latter being produced by an enzymatic reaction which could not be mimicked *in vitro*. The isolation of the three new compounds **9**, **12** and **14** together with the earlier reports of the isolation of photogedunin [9, 12] and limonexic acid [13], indicate that these structures are much more widespread in Nature than previously believed. We refrained from naming the compounds **12** and **14** as photo-products, since to our belief the reaction involving their formation is more of an enzymatic oxidation than a simple photolytic process taking place in the plant. The word photo is retained in the name of **9** since the corresponding acetate, photogedunin, has been reported as such.

EXPERIMENTAL

Mp's are uncorrected. IR spectra were recorded as KBr pellets. NMR spectra refer to 5–10% solns in $CDCl_3$, using TMS as internal standard. MS were recorded under the direction of Dr. Z. V. I. Zaretskii, and the relative intensities of the peaks, given in parentheses, are reported with reference to the most intense peak taken as 100%. Si gel 60 (E. Merck) 70–230 mesh was used for column chromatography. TLC were carried on chromatoplates of Si gel F. For PLC a thick layer (1·0 mm) of Si gel PF_{254} was used. Acetylations were carried out with Ac_2O–pyridine at room temp. for 20 hr and worked up by removal of the reagents under red. pres. on a hot H_2O bath.

Extraction and isolation procedure. Ground seeds of *Cabralea eichleriana* DC. were successively extracted with hot petrol, and $CHCl_3$ in a Soxhlet. (a) Concentration of the petrol extract gave

an oil (50 g) which was chromatographed on a Si gel column (1300 g) and eluted with *n*-hexane followed by mixtures of hexane with increasing quantities of Et_2O. Fractions of about 250 ml were collected. Most of the fractions eluted up to *n*-hexane–Et_2O (1:1) were oils or low melting solids having a paraffinic character, and were therefore not further investigated.

Hexane–Et_2O (1:1) eluted a mixture (fr. 53–55, 300 mg) which upon separation using preparative chromatoplates (EtOAc–C_6H_6 3:7) yielded cabraleone (**1**) (30 mg) as colorless needles (*n*-hexane), mp and mmp with an authentic sample 163–166°. Further elution with the same solvent mixture gave a product (fr. 71–77) which crystallized from *n*-hexane to give cabraleadiol (**2**) (300 mg), m.p. and m.m.p. with an authentic sample 176–178°.

(b) The $CHCl_3$ extract upon evaporation under reduced pressure left a viscous residue (13 g) which was chromatographed on a Si gel H column (300 g) using slight pressure, and eluted with C_6H_6–$CHCl_3$ mixtures. Fractions of 20 ml each were collected and monitored by TLC and NMR. Fractions which appeared to be identical were combined. The various compounds isolated from these fractions are listed below.

Eluent	Fraction	Compounds	Mp
C_6H_6:$CHCl_3$ 1:1	65–66	methyl angolensate (**8**) (0·04 g)	201–203
C_6H_6:$CHCl_3$ 1:1	77–78	gedunin (**5**) (0·12 g)	216–218
C_6H_6:$CHCl_3$ 1:1	79–84	7-deacetoxy-7-oxogedunin (**6**) (0·23 g)	261–263
C_6H_6:$CHCl_3$ 1:1	106–115	7-deacetoxy-7-hydroxygedunin (**7**) (0·26 g)	250
C_6H_6:$CHCl_3$ 1:1	120–125	fissinolide (**3**) (0·08 g)	170–172
C_6H_6:$CHCl_3$ 1:2	301–360	3-deacetylfissinolide (**4**) (1·20 g)	192–194
EtOAc		mixture (3·1 g)	

All known compounds mentioned above were characterised by their NMR spectral data and compared with authentic samples.

The mixture eluted with EtOAc was rechromatographed on Si gel and the column eluted with a solvent mixture of EtOAc–$CHCl_3$ (1:1). The fractions (15 ml each) were monitored by TLC and when required by NMR. They were combined to 4 main fractions A (0·2 g), B (0·5 g), C (0·25 g) and D (0·3 g). Fraction B was a mixture which could not be resolved.

7-Deacetoxy-7-hydroxyphotogedunin (**9**). During concentration of fraction A a colorless crystalline compound separated, which was collected by filtration and recrystallized (boiling (MeOH) to give **9** mp 284–287° (dec.), insoluble in $CHCl_3$, Et_2O, $(CH_3)_2CO$ and MeOH, but soluble in DMSO and pyridine; ν_{max} 3486, 3266, 1758, 1728, 1656, 1650, 1264, 1206, 1023, 1011, 935 and 923 cm^{-1}. MS *m/e* 472 M^+ (very small), 454 (1·2), 428 (7·0), 410 (6·5), 311 (8·7), 298 (16·6), 163 (12·2), 161 (11·8), 149 (15·3), 147 (26·7), 137 (36·7), 121 (26·7), 119 (21·8), 109 (19·6), 105 (24·0), 93 (25·8), 91 (36·7), 79 (24·0), 77 (24·0), 69 (23·3), 55 (27·1), 44 (100) and 43 (30). (Found: C, 66·01; H, 6·80; M^+ 472. $C_{26}H_{32}O_8$ requires: C, 66·08; H, 6·83%; M.W. 472·52).

Acetylation of (**9**). Usual acetylation of **9** (40 mg) induced the appearance of a red color in the solution, which remained for 20 hr. Following work-up the reaction product was separated on preparative chromatoplates (EtOAc–C_6H_6 1:1) to yield 2 compounds: (i) *7-deacetoxy-7-hydroxy-23-acetylphotogedunin* (**10**) (10 mg) mp 195–200° (from MeOH-CH_2Cl_2); ν_{max} 3478, 1788–1733, 1661, 1259, 1200 and 1028 cm^{-1}. MS *m/e* 514 M^+ (0·1), 470 (0·8), 428 (0·9), 410 (3·6), 392 (1·5), 311 (5·8), 298 (16·5), 149 (7·8), 147 (8·6), 137 (16·1), 105 (8·7), 93 (8·7), 91 (11·2), 79 (8·7), 77 (9·3), 68 (8·1), 60 (39·1), 44 (100) and 43 (60·1). (ii) *Photogedunin acetate* (**11**) with the higher R_f (12 mg) mp 235–242° (from MeOH–CH_2Cl_2) [lit. [9] mp 286–296° (dec.)]; ν_{max} 1788–1732, 1663, 1258, 1236, 1202, 1034 and 1023 cm^{-1}. MS *m/e* 556 M^+ (5·4), 496 (4·6), 137 (7·8), 135 (7·8), 121 (16·7), 109 (10·6), 108 (8·2), 93 (11·0), 91 (11·0), 83 (9·8), 81 (10·2), 79 (9·2), 71 (9·6), 69 (21·6), 67 (8·9), 57 (16·7), 55 (20·6), 44 (14·9), 43 (100).

Cabralin (**12**). Fraction C after evaporation of the solvents crystallized from an Et_2O–petrol mixture to give **12** as colorless crystals, mp 140–146° (dec.); ν_{max} 3400, 1762–1713, 1293, 1229, 1192, 1136, 1034, 1010 and 922 cm^{-1}. MS, *m/e* 544 M^+ (6·5), 526 (0·8), 500 (0·9), 484 (18·8), 411 (10·7), 373 (16·1), 355 (27·9), 208 (14·4), 149 (33·9), 141 (22·1), 119 (24·4), 105 (19·4), 91 (19·4), 69 (17·9), 55 (24·2), 44 (47·6) and 43 (100). Acetylation of **12** gave *cabralin acetate* (**13**) mp 147–152 (dec.) (CH_2Cl_2–petrol). ν_{max} 1783–1713, 1326, 1293, 1232, 1190, 1138, 1036, 1028, 1000, 993, 967 and 891 cm^{-1}. MS *m/e* 586 M^+ (3·3), 526 (8·7), 373 (7·7), 355 (16·5), 149 (16·5), 141 (12·1), 119 (13·6), 105 (10·4), 91 (10·0), 81 (7·9), 79 (6·7), 69 (12·5), 60 (33·3), 55 (13·7), 45 (21·7), 44 (27·5) and 43 (100). (Found: C, 63·08; H, 6·51; M^+ 586. $C_{31}H_{38}O_{11}$ requires: C, 63·47; H, 6·53%; MW. 586·61).

Isocabralin acetate (**15**). Fraction D was acetylated at room temp. A similar development of a red color, as in the acetylation of **9** was observed. Upon separation on preparative chromatoplates (EtOAc–Et_2O 3:2), the mixture afforded *cabralin acetate* (**13**) (0·06 g) identical with that obtained by the acetylation of **12** and *isocabralin acetate* (**15**) (0·2 g), mp 137–140° (CH_2Cl_2–petrol); ν_{max} 1803-1714, 1296, 1230, 1200, 1174, 1145, 1040, 1018, 996 and 885 cm^{-1}. MS *m/e* 586 M^+ (1·1), 542 (0·6), 526 (4·3), 466 (5·8), 355 (23·0), 149 (19·6), 141 (14·2), 137 (10·4), 135 (11·5), 121 (13·1), 119 (19·2), 107 (10·0), 105 (16·5), 95 (11·9), 93 (11·5), 91 (20·0), 81 (13·9), 79 (13·0), 69 (23·5), 67 (14·2), 60 (14·6), 55 (27·7), 44 (50·0), and 43 (100). (Found: C, 63·70; H, 6·68; M^+ 586. $C_{31}H_{38}O_{11}$ requires: C, 63·47; H, 6·53%; M.W. 586·61).

Photolytic oxidation of fissinolide (**3**). A solution of fissinolide (1 g) in C_6H_6 (150 ml) in a Pyrex flask and under a stream of O_2 was irradiated with a UV lamp (Hanovia 450 W). After disappearance of the fissinolide (6 hr), as shown by TLC, the solvent was evaporated and the residue subjected to chromatography on Si gel. Elution with Et_2O gave cabralin (**12**) (300 mg): mp and mmp with **12**, obtained from fraction C, 140–146° (dec.).

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