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CHROMANONE ACIDS IN *CALOPHYLLUM BRASILIENSE* SEED OIL*

R. D. PLATTNER, G. F. SPENCER, D. WEISLEDER and R. KLEIMAN

Northern Regional Research Laboratory,† Peoria, IL 61604, U.S.A.

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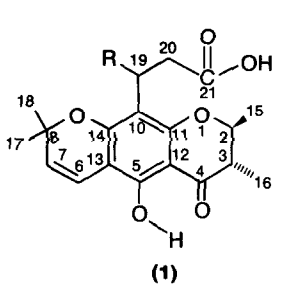
Key Word Index—*Calophyllum brasiliense*; Guttiferae; isoapetalic acid; apetalic acid; blancoic acid; GC–MS; ^{13}C NMR.

Abstract—Three homologous *trans* chromanone carboxylic acids (**1a**, **1b** and **1c**) made up about 20% of the pentane–hexane extract from *Calophyllum brasiliense* seed kernels. The *cis* isomers (**2a**, **2b** and 2c) were also present but only in trace amounts. Four of these six chromanone acids (**1a**, **1b**, **1c** and **2a**) were isolated as methyl esters by countercurrent distribution. Compounds **1b**, **2a**, **2b** and **2c** are new; compounds **1a** and **1c** are the previously reported isoapetalic acid and blancoic acid, respectively.

INTRODUCTION

IN A CONTINUING survey of plant seeds collected from many parts of the world,[1] we found large amounts of unusual components in the seed oil of *Calophyllum brasiliense* Camb. (Guttiferae). Various workers have reported isolating many 4-phenylcoumarins, 4-alkylcoumarins and chromanone carboxylic acids from the bark and seeds of a number of *Calo-*



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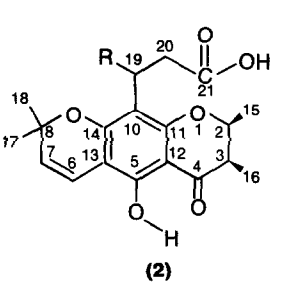

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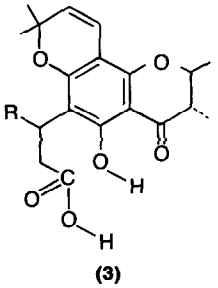

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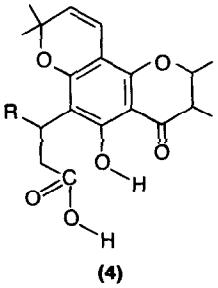

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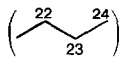
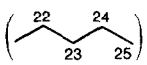
(a) R = propyl  (b) R = *n*-butyl  (c) R = *n*-pentyl

* Presented at the 47th fall meeting of the American Oil Chemists' Society, Chicago, Ill., Sept. 1973.

† Agricultural Research Service, U.S. Department of Agriculture.

[1] BARCLAY, A. S. and EARLE, F. R. (1974) *Econ. Bot.* In press.

phyllum species.[2-12] Application of GLC and GC–MS to fractions of *C. brasiliense* seed oil has now demonstrated the presence of *trans* and *cis* chromanone series, each containing three homologous components (**1**a, **1**b, **1**c, **2**a, **2**b, **2**c).

RESULTS AND DISCUSSION

The *C. brasiliense* seed oil contained 13% free fatty acids and 20% chromanone carboxylic acids. Two distinct classes of the latter were indicated by TLC, with the major class being slightly more mobile. GLC and GC–MS of the corresponding two chromanone fractions isolated by preparative TLC showed that each contained a series of three homologs (**1**a, **1**b, **1**c and **2**a, **2**b, **2**c). Spectra of corresponding compounds in the two series were identical; base peaks (M-15) and molecular ions were the only intense signals. However, small peaks were observed representing ions due to the loss of CH_2COOH (M-56) from each acid or CH_2COOMe (M-73) for its methyl ester. Furthermore, ions representing the loss of propyl (M-43), butyl (M-57) and pentyl (M-71) side chains in the three respective component pairs were observed (Table 1).

TABLE 1. OPTICAL ROTATION, MASS SPECTRAL AND YIELD DATA FOR *Calophyllum brasiliense* SEED OIL COMPONENTS AND DERIVATIVES

Compound	Whole seed oil (%)	$[\alpha]_D^{25}$*	Molecular weight	*m/e*§
1a	12·3	—	388	388 (25), 373 (100), 329 (11)
2a	0·3	—	388	
1b	7·2	—	402	402 (25), 387 (100), 343 (11)
2b	0·1	—	402	
1c	1·0	—	416	416 (25), 401 (100), 357 (11)
2c	Tr	—	416	
Methyl ester of:				
1a	—	−101°†	402	402 (22), 387 (100), 359 (5), 329 (9), 327 (3)
2a	—	−51°	402	
1b	—	−85	416	416 (21), 401 (100), 359 (5), 343 (7), 327 (7)
2b	—	—	416	
1c	—	−72°‡	430	430 (24), 415 (100), 359 (5), 355 (6), 327 (3)
2c	—	—	430	
1a-acetate	—	—	444	444 (6), 402 (25), 387 (100), 329 (8)

* 2% $CHCl_3$ solution.
† Reported −68·3°[2] in $CHCl_3$.
‡ Reported −66·7°[3] in $CHCl_3$ for **1**c (blancoic acid).
§ Relative intensities in parentheses – base peak = (100).

[2] GUERREIRO, E., KUNESCH, G. and POLONSKY, J. (1971) *Phytochemistry* **10**, 2139.
[3] STOUT, G. H. and SEARS, K. D. (1969) *J. Org. Chem.* **34**, 4203.
[4] GOVINDACHARI, T. R., PRAKASH, D. and VISWANATHAN, N. (1968) *Tetrahedron* **24**, 6411.
[5] GOVINDACHARI, T. R., PRAKASH, D. and VISWANATHAN, N. (1967) *Tetrahedron Letters* **42**, 4177.
[6] GUERREIRO, E., KUNESCH, G. and POLONSKY, J. (1973) *Phytochemistry* **12**, 185.
[7] STOUT, G. H., KRAHN, M. and BRECK, G. D. (1968) *Tetrahedron Letters* **29**, 3285.
[8] POLONSKY, J. (1958) *Bull. Soc. Chim. Fr.* **1079**, 929.
[9] NIGAM, S. K. and MITRA, C. R. (1967) *Tetrahedron Letters* **28**, 2633.
[10] STOUT, G. H. and STEPHENS, K. L. (1968) *J. Org. Chem.* **29**, 3604.
[11] STOUT, G. H., HICKERNELL, G. K. and SEARS, K. D. (1968) *J. Org. Chem.* **33**, 4191.
[12] BRECK, G. D. and STOUT, G. H. (1969) *J. Org. Chem.* **34**, 4203.

Isomeric esters of the two series were not readily distinguishable by GLC although those of the minor fraction were slightly more mobile on Apiezon L columns. Within each series, the homologous acids (i.e. **1**a, **1**b and **1**c for the major series; **2**a, **2**b and **2**c for the minor one) were present in the ratio 12:7:1. Based on preparative TLC, the major series made up 98% of the total chromanones.

TABLE 2. NMR CHEMICAL SHIFTS (ppm) AND COUPLING CONSTANTS (Hz) FOR *C. brasiliense* SEED OIL COMPONENTS AND DERIVATIVES*

Compound type	H-2	H-3	Me-2	Me-3	H-6	Me-8	H-11	H-12	O H	OAc	O ‖ CO–H	O ‖ C–OCH$_3$	CH_2CH_3	O ‖ C–O–C–CH$_3$
I†	4·11*m*	2·50*m*	1·49*d* *J* 6	1·19*d* *J* 7	6·59*d* *J* 10	1·41*s*	3·68*m*	2·72*m*	12·45*s*		9·1*s*		0·84*t*	
I-Me‡	4·11*m*	2·50*m*	1·49*d* *J* 6	1·19*d* *J* 7	6·59*d* *J* 10	1·41*s*	3·68*m*	2·72*m*	12·45*s*			3·57*s*	0·84*t*	
I-Me:Ac‡	4·11*m*	2·5*m*	1·49*d* *J* 6	1·19*d* *J* 7	6·32*d* *J* 10	1·41*s*	3·68*m*	2·75*d*		2·37*s*		3·57*s*	0·84*t*	
I-Anh†	4·11*m*	2·5*m*	1·49*d* *J* 6	1·19*d* *J* 7	6·59*d* *J* 10	1·41*s*	3·68*m*	2·70*m*	12·47*s*				0·84*t*	2·09*s*
2-Me†	4·51*m*	2·5*m*	1·34*d* *J* 6	1·12*d* *J* 7	6·58*d* *J* 10	1·41*s*	3·70*m*	2·70*m*	12·45*s*			3·57*s*	0·84*t*	
2a-Me	4·51*m*	2·5*m*	1·34*d* *J* 6	1·12*d* *J* 7	6·58*d* *J* 10	1·41*s*	3·70*m*	2·70*m*	12·45*s*			3·57*s*	0·84*t*	
2a-Me:Ac	4·51*m*	2·5*m*	1·34*d* *J* 6	1·12*d* *J* 7	6·32*d* *J* 10	1·41*s*	3·70*m*	2·70*m*		2·37*s*		3·57*s*	0·84*t*	

* Me = methyl ester; Me:Ac = methyl ester acetylated at C-5; Anh = mixed anhydride of chromanone acid and acetic acid.

† Mixture of homologs a, b and c.

‡ Individual homologs, a, b and c, give same signals.

Proton NMR data (Table 2) for the methyl esters of the major TLC fraction are similar to those reported for isoapetalic acid (**1**a)[2] and blancoic acid (**1**c),[3] whereas that of the minor fraction resembled those reported for apetalic acid (**2**a without specification of absolute stereochemistry at C-2 and C-3)[4,5] and isocalolongic acid (**4**a).[6] A multiplet at $\delta = 4{\cdot}50$ (1H) in the minor fraction compared to one at $\delta = 4{\cdot}11$ (1H) in the major fraction is the only significant difference between the two. These signals indicate that the 2- and 3-methyl groups are *trans* (either structures **1** or **3**) in the major fraction and *cis* (**2** or **4**) in the minor one.

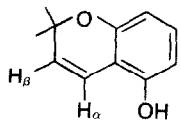
The *trans* series of homologs were separated by preparative GLC of their methyl esters. However, the recovered material was shown by TLC and proton NMR to be an almost equal mixture of *cis* and *trans* isomers, although the starting material was nearly pure *trans*. Apparently epimerization occurred at the easily enolized hydrogen α to the carbonyl of the chromanones.[11] To avoid such epimerization, the methyl esters were separated by countercurrent distribution (CCD).

Acetylation causes marked changes in the chemical shifts of Hα in compounds like **5** but not **6**.[13] Comparison of H-6 data in Table 2 for esters acetylated at C-5 with that for those unacetylated clearly supports the assigned structures. NMR of the methyl esters of **1**a, **1**b and **1**c were virtually indistinguishable, except for the area of the broad multiplet ($\delta = 1{\cdot}65$) resulting from side-chain methylene protons. This lack of differences in the spectra indicates that all the side chains are unbranched. Comparison of ^{13}C NMR spectra of the methyl esters of **1**a and **1**b with those of model hydrocarbons,[14] shows that data

[13] ARNONE, A., CARDILLO, C., MERLINI, L. and MONDELLI, R. (1967) *Tetrahedron Letters* **43**, 4201.

[14] LINDEMAN, L. P. and ADAMS, J. Q. (1971) *Anal. Chem.* **43**, 1245.

for carbons 22–24 (**1a**) and 22–25 (**1b**) are in good agreement with an *n*-propyl and *n*-butyl group, respectively, rather than an alternative branched structure for either (Table 3).[15]



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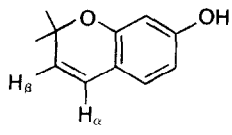

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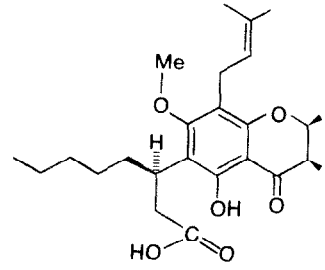

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Treatment of the mixture of chromanone carboxylic acids with acetic anhydride and pyridine at room temperature as described by Guerreiro *et al.*[2] did not produce the lactones that would have been expected from structures **3** and **4**. The product, which formed under these conditions, but decomposed readily back to the free acid, was not an acetate but probably a mixed anhydride of a chromanone carboxylic acid with acetic acid. NMR and IR were consistent with this assignment. The failure to form a lactone confirms that the compounds are types **1** and **2**.

TABLE 3. ^{13}C NMR SIGNALS (ppm) AND MULTIPLICITIES DUE TO C–H COUPING FOR *C. brasiliense* SEED OIL COMPONENT METHYL ESTERS[15]

Carbon	1a	1b
C-2	79·0*d*	78·9*d*
C-3	45·9*d*	45·8*d*
C-4	199·5	199·3
C-5 (or C-11 or C-14)	157·2*s* (100·0*s*)	157·0*s* (160·0*s*)
C-6 (or C-7)	116·0*d* (125·7*d*)	115·7*d* (125·5*d*)
C-8	78·3*s*	78·1*s*
C-10 (or C-12 or C-13)	102·0*s* (102·8*s*, 109·5*s*)	101·8*s* (102·6*s*, 109·3*s*)
C-15	19·7*q*	19·5*q*
C-16	10·7*q*	10·5*q*
C-17 (or C-18)	28·5*q* (28·5*q*)	28·3*q* (28·4*q*)
C-19	30·8*d*	30·8*d*
C-20	38·9*t*	38·7*t*
C-21	173·7*s*	173·6*s*
C-22	35·8*	33·0*t*
C-23	21·1*	30·0*t*
C-24	14·3*q*	22·6*t*
C-25	(1b only)	14·0*q*
OMe	51·4*q*	51·2*q*

* Multiplicity uncertain.

GLC, GC–MS and TLC of the pentane–hexane extract of ground seed hulls showed the same six unusual components present in seed kernel oil.

Comparison of the CD spectrum of the methyl ester of **2a** after hydrogenation with derivatives of isopapuanic acid (**7**)[11] clearly established the sterochemistry of the chromanone portion of **2a** as 2*S*,3*R*.[16] The optical rotations reported for the methyl ester of apetalic acid in the literature are $+30{\cdot}4^{4,5}$ and $+13{\cdot}4^{6}$ (for $CHCl_3$ solutions). The value we obtained

[15] HIGHET, R. J. Private communication.
[16] STOUT, G. H. Private communication.

for the methyl ester of compound **2a** is −51° (Table 4). Therefore, our **2a** is not the enantiomer of the previously reported apetalic acid[4–6] and must differ in the relative stereochemistry of C-19 vs C-2 and C-3. ORD curves for the esters of **1a**, **1b** and **2c** (Table 3) were identical in shape and sign of Cotton effects, differing only in the magnitude of the rotation. Breck has shown that for methyl esters of **3c** and **4c** the sign of all Cotton effects is dependent on the configuration of C-2.[17] On this basis **1a**, **1b** and **2a** must have the same stereochemistry at C-2. Therefore, **1a** and **1b** differ from **2a** at C-3 and so are 2*S*,3*S*. Consequently, the acids isolated from the seed oil as methyl esters by CCD are isoapetalic acid (**1a**), a homolog (**1b**) of isoapetalic acid, blancoic acid (**1c**) and (2*S*,3*R*)-apetalic acid (**2a**). The possibility exists that the *cis* isomers arose from the *trans* series,[11] but our use of CCD rather than preparative GLC of Na_2CO_3-treated material was designed to minimize this epimerization. Since methyl esters of **2b** and **2c** were not separated by CCD, optical data were not determined for them; so we cannot specify their stereochemistry.

TABLE 4. ORD DATA FOR *C. brasiliense* SEED OIL COMPONENT METHYL ESTERS

Compound	Concentration* (mg/ml)	Specific rotation† at selected wavelengths (nm) 589	385	340	325	310	295
1a	0·047	−101	−820	−550	−750	+580	−650
1b	0·026	−85	−800	−420	−850	+800	−904
2a	0·012	−51	−500	−340	−750	+1650	+140

* In $CHCl_3$ solution. † At 26°.

Isoapetalic acid and blancoic acid have been previously reported in bark or seeds of other *Calophyllum* species.[8,9] However *n*-butyl isomers have not previously been described, and our report is the first of a homologous series of acids being present in one species of *Calophyllum*. The only other such report in the family Guttiferae, to our knowledge, was by Govindachari and co-workers[18] who found both a 4-propyl and a 4-isobutyl substituent in 4-alkylcoumarins from *Mesua ferra* bark.

EXPERIMENTAL

Seed kernels (60%) were separated from hulls. Each was ground and extracted with pentane–hexane in Soxhlet extractors for 24 hr. The seed kernels contained 76% oil; the hulls, 7%. Free acids (33% of the whole oil) were separated from neutral components by washing a portion of the oil with a 5% Na_2CO_3 soln, acidifying the washings and extracting with ether. TLC (hexane–ether, 3:2) of the free acids gave a broad band of unusual components in addition to fatty acids. After esterification in Et_2O–MeOH with CH_2N_2 the broad band was resolved into two completely resolved spots (R_f 0·40 and 0·45) which could be more easily separated by preparative TLC.

The GLC columns for the oils and free acids were 180 × 0·32 cm stainless steel packed with a nonpolar phase (OV-1 or Dexsil 300) and were programmed from 200 to 400° at 4°/min. Methyl esters and acetates were analyzed on a 122 × 0·64 cm glass column packed with 5% Apiezon L and operated at 200°. Preparative GLC separations were made on a 305 × 0·96 cm glass column packed with 5% Apiezon L. MS were taken with either a probe inlet or a gas chromatograph coupled to a Du Pont 21-492-1 mass spectrometer. Glass columns packed with 5% Apiezon L or stainless steel columns packed with OV-1 or Dexsil 300 were used in GC–MS analyses. Optical rotations were determined at the sodium D line from 2% $CHCl_3$ soln. ORD curves were measured with a Carey Model 60 spectropolarimeter. IR spectra were recorded from thin films on NaCl disks. Proton NMR spectra were obtained from $CDCl_3$ soln with a Varian HA-100 instrument. For ^{13}C NMR the same solvent and internal standard (TMS) were employed, and the instrument was a Varian XL-100 equipped with a Digi-Lab Fourier

[17] BRECK, G. D. (1971) *Diss. Abst. Int.* **31**, 3909B.

[18] GOVINDACHARI, T. R., PAI, B. R., SUBRAMANIAM, P. S., RAO, U. R. and MUTHAKUMARASWAMY, N. (1967) *Tetrahedron* **23**, 4161.

Transform accessory. Off resonance decoupling provided spectra with signals for each carbon atom having multiplicity indicating the number of attached hydrogen atoms.

Individual components were isolated from the whole seed oil by CCD as follows: The chromanone acids were concentrated by distributing 18 g of the oil between equal volumes of hexane and acetonitrile. The acetonitrile fraction (4 g) was methylated with CH_2N_2 in Et_2O–MeOH, and the esters were separated in a 200-tube (40 ml per phase) Craig CCD apparatus charged with equal volumes of equilibrated acetonitrile and hexane. The extent of separation was monitored after 200, 500 and 1000 transfers by using the contents of every 10th tube for TLC, GLC and weight recovery. After 200 and 500 transfers, tubes not containing chromanone esters were removed. The apparatus was set to transfer tube 199 to tube 0 and distribution was continued.

After 1000 transfers, separation of components was incomplete, but some tubes contained pure materials as evidenced by TLC and GLC. These tubes were combined to obtain pure esters of **2**a, **1**a, **1**b and **1**c (in order of increasing mobility). Tubes containing unresolved esters of **2**b and **2**c were between those with **2**a and **1**a. MS and NMR data for mixtures and isolated componets are given in Tables 1 and 2. UV λ_{max}^{EtOH} values (225, 264, 274, 310 nm) and IR absorbances for methyl esters of **1**a, **1**b, **1**c and **2**a were indistinguishable from those reported in the literature for the methyl ester of isoapetalic acid.[2]

Acetylation of esters of **1***a*, **1***b*, **1***c and* **2***a*. Individual esters (30–50 mg) were dissolved in 2 ml Ac_2O and 1 ml pyridine and heated under reflux on a steam bath for 5 hr. Excess reagents were removed under N_2, and samples were purified by TLC. Yields were about 80%. Mass spectral and NMR data are given in Tables 1 and 2. IR: 1800, 1740, 1650, 1630 cm^{-1}.

Attempted lactonization of free acids. Mixed chromanone acids (50 mg) were dissolved in 100 μl Ac_2O and 50 μl pyridine and left at room temp. for 0·5 hr. Solvents were removed under N_2 at room temp. Attempts to purify the product by TLC resulted in isolation of the original mixture of free acids. However, examination of the crude preparation by NMR, IR, GLC and GC–MS immediately after solvent removal showed that a reaction product had formed. NMR data appears in Table 2. IR: 1825, 1750, 1650, 1630 cm^{-1}. MS: 370 (M-60)–22%; 355–100%; 313–14%.