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Two chromene derivatives from *Calyptranthes tricona*☆

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Abstract

Two α-monomethyl chromene derivatives were isolated from the leaf essential oil of *Calyptranthes tricona* from Brazil which were characterized by ^{1}H- and ^{13}C-NMR. Besides these components, which represent about half of the oil, classical terpenoid structures could be identified, among which *cis*-β-farnesene is the most abundant (26.6%). A biosynthetic pathway could be proposed to explain the formation of the chromene derivatives in the plant. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The genus *Calyptranthes*, with about 100 species identified in Tropical America from Mexico to Uruguay (Mc Vaugh, 1968) belongs to the Myrtaceae family. Six species were identified in Rio Grande do Sul (Brazil) (Marchiori & Sobral, 1997): *C. concinna* DC, *C. grandifolia* Berg, *C. lucida* Martius ex DC, *C. pileata* Legrand, *C. rubella* (Berg) Legrand and *C. tricona* Legrand.

As part of our continuous interest in the essential oils of Brazilian native plant, we had analyzed those obtained from leaves of two species of *Calyptranthes: C. concinna* and *C. grandifolia*. Both samples were very different, the former being exclusively terpenic with predominance of pinenes (85.9%) and β-caryophyllene (10.5%), while the latter contained a high amount of elemicine (76%) (Menut et al., 1997). To our knowledge, the only other studies relative to this genus were performed on another brazilian species, *C. spruceana* (da Silva, Luz, Zoghbi, Ramos & Maia, 1984). The authors distinguished two chemical varieties, which were characterized by high contents of limonene, geranial and perillaldehyde for the variety A while pinenes and citral for the variety B.

We present here, the results of our chemical investigations on the leaf essential oil of a third species, *C. tricona*, collected in Tenente Portela area (Rio Grande do Sul, Brazil).

The composition of this oil is quite different from those previously obtained from the other species of *Calyptranthes*; furthermore, we have isolated and characterized two new chromene derivatives for which an original biosynthetic pathway could be proposed.

2. Results and discussion

Among the 20 compounds found above 0.1% in the volatile extracts of leaves of *C. tricona*, 18 were terpenoids usually encountered in essential oils and their identification was performed directly by GC and GC-mass spectrometry (GC-MS) (Table 1). In this chemical class, which represents nearly half of the whole oil,

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Table 1
Identified constituents and approximate percentage in the leaf essential oil of *Calyptranthes tricona*

No.	Component	Area percentage	RI[a]	Identification method
1	α-copaene	0.3	1378	RI, C[b], MS[c]
2	β-elemene	0.3	1389	RI, MS
3	α-gurjunene	0.2	1411	RI, MS
4	β-caryophyllene	0.6	1420	RI, C, MS
5	*cis*-β-farnesene	26.6	1447	RI, MS
6	α-humulene	0.3	1454	RI, C, MS
7	*cis*-β-santalene	0.3	1466	RI, MS
8	bicyclogermacrene	5.7	1494	RI, MS
9	δ-cadinene	1.0	1518	RI, MS
10	β-calacorene	0.3	1535	RI, MS
11	nerolidol	0.8	1554	RI, C, MS
12	ledol	0.2	1564	RI, MS
13	spathulenol	2.7	1573	RI, MS
14	caryophyllene epoxide	0.6	1578	RI, C, MS
15	globulol	1.8	1583	RI, C, MS
16	epiglobulol	1.4	1590	RI, MS
17	T-cadinol	0.6	1636	RI, MS
18	α-cadinol	1.4	1649	RI, MS
19	chromeneI	19.7	1664	MS, NMR
20	chromene II	28.1	1729	MS, NMR

[a] RI: Linear retention indices on OV_{101} column.
[b] C: Co-chromatography with authentic samples.
[c] MS: Identification based on mass spectral data.

an acyclic sesquiterpene, *cis*-β-farnesene predominates (26.6%) accompanied by bicyclogermacrene (5.7%).

The other part of the mixture composed of only two constituents (**19**, $MW = 206$ and **20**, $MW = 220$) characterized by linear retention indices higher than 1660 on OV_{101}. Examination of their mass spectra indicated two related aromatic structures differentiated by the presence of a methyl group, but which could not be directly identified.

After fractionation of the essential oil on a silica column, two fractions enriched in **19** and **20** were obtained and studied by ^{1}H and ^{13}C-NMR (Table 2).

Table 2
NMR data ($CDCl_3$,; δ, ppm) for Chromene I (**19**) and chromene II (**20**)

H or C	Chromene I		Chromene II	
2	4.91 *m*	71.9	4.90 *m*	71.6
3	5.43 *dd* (9.8, 3.1)	122.2	5.47 *dd* (10.0, 3.1)	122.6
4	6.70 *ddd* (9.8, 1.8, 0.5)	119.1	6.60 *dd* (10.0, 1.8)	119.5
5		156.6		153.0
6	6.02 *d* (2.3)	92.1	6.04 *s*	88.4
7		159.0		161.4
8	6.05 *dd* (2.3, 0.5)	94.1		106.7
9		154.1		155.7
10		105.2		105.2
11	1.42 *d* (6.6)	21.4	1.41 *d* (6.6)	21.4
12	3.78 *s*	55.9	3.80 *s*	56.1
13	3.75 *s*	55.7	3.84 *s*	56.0
14			1.99 *s*	8.0

The observation of the DEPT, COSY and HETCOR spectra allowed to specify the structures of components **19** and **20**. They are chromenes which are particular as they bear only one CH_3 group in position 2.

All these propositions were unambiguously confirmed by chemical synthesis of compound **19** according to the reaction scheme described in Fig. 1: esterification by crotonyl chloride, followed by a Fries transposition under the action of $AlCl_3$; the benzopyranone thus obtained is finally reduced to alcohol which is dehydrated and leads, with a 12% global yield, to a compound exhibiting the same spectral characteristics as the natural product **19**.

The benzopyranic structures identified in the *Calyptranthes tricona* essential oil are very special, as they differ from "classical" structures for natural chromenes by the presence of only one methyl group in α-position to the oxygen atom of the heterocycle. Indeed, in the Asteraceae family for example, one can find a great variety of chromenes characterized by a gem-dimethyl α to the heteroatom and differing by the nature and the position of the substituents on the aromatic ring (Proksch & Rodriguez, 1983); one of the simplest representatives of this class is demethoxyageratochromene (precocene 1) identified in species of the *Ageratum* genus (Menut, Lamaty, Amvam Zollo, Kuiaté & Bessière, 1993).

In terms of biosynthesis, the position of oxygenated groups on the aromatic ring of all the chromenes, indicates that these products are issued from a polyketide

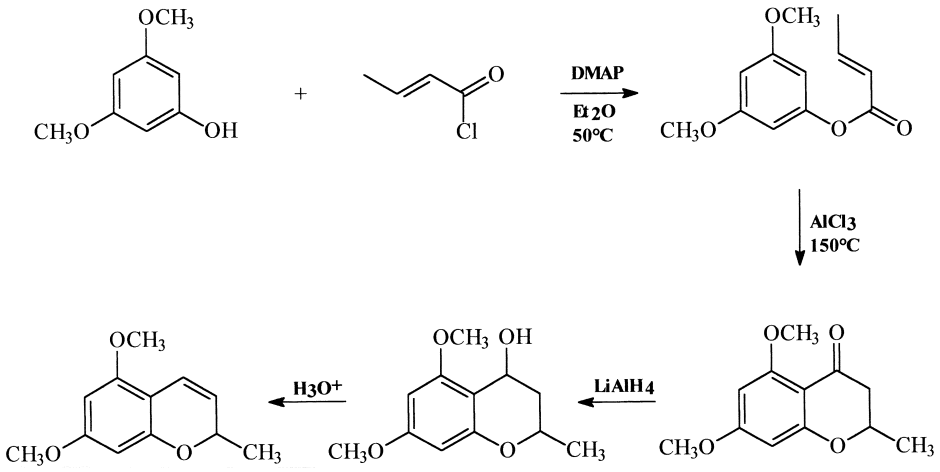
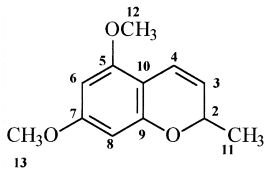


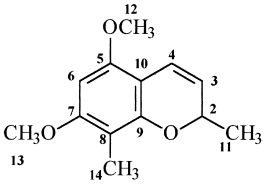
Fig. 1. Chemical synthesis of chromene I (**19**).

pathway, that is to say, according to the same metabolic pathway as phloroacetophenone and eugenone (Mann, 1987). In the case of classical chromenes, a prenylation is indispensable; it is followed by a cyclization with a phenol function situated in ortho to give the α,α-dimethylpyranic structure; the α-mono-methyl structure of *C. tricona* chromenes probably results from the direct cyclization between the acyl residue and the ortho phenol function (Fig. 2).

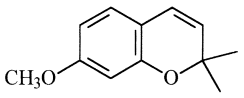
This important result, on the chemotaxonomic level, justifies further investigations in the Myrtaceae family. The identification of structures of the same type in other species of this family allowed to reinforce the hypothesis of the new metabolic process previously proposed.



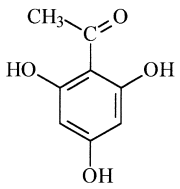
19



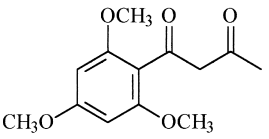
20



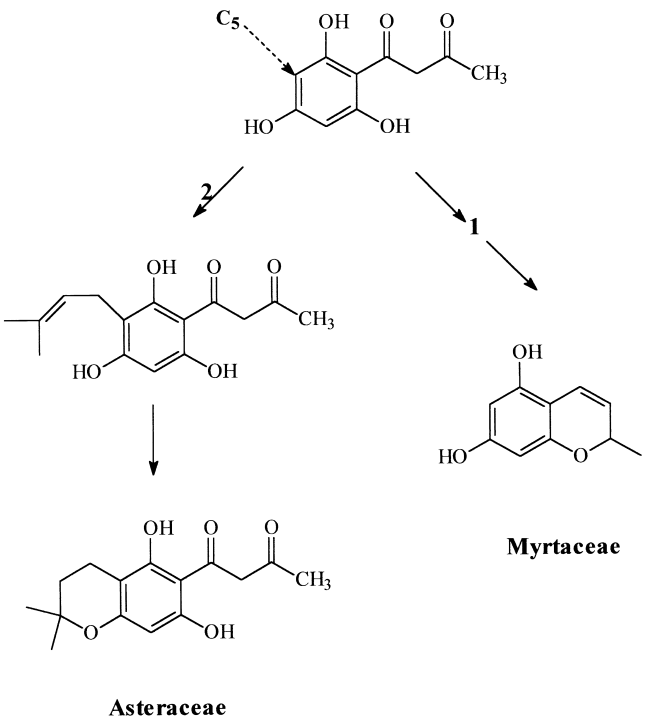
Precocene I



Phloroacetophenone



Eugenone



Myrtaceae

Asteraceae

Fig. 2. Schematic representation of two representative chromene derivatives biosynthesis.

3. Experimental

3.1. Material

Leaves of *Calyptranthes tricona* were collected in Tenente Portela area (Rio Grande do Sul, Brazil) in April 1993. The botanical identification of the material

collected was carried out by M. Sobral and a voucher specimen was deposited in the Herbarium of the Instituto de Biociências, UFRGS (Sobral 6583 ICN). The oil was obtained in 0.18% (w/w) yield from fresh plant material by hydrodistillation using a Clevenger type apparatus for 5 h and analyzed by GC and GC-MS.

3.2. Capillary GC

The oil samples were analyzed on a Shimatzu GC 14A with flame ionization detectors fitted with two fused silica capillary columns (25 m × 0.25 mm i.d. coated with OV_{101} and 25 m × 0.22 mm i.d. coated with Carbowax 20 M). Temperature programme 50–200°C at 5°C/min, injector temperature 220°C, detector temperature 250°C, carrier gas N_2 0.8 ml/min. The linear retention indices of the components were determined relatively to the retention times of a series of *n*-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

3.3. GC-MS

The essential oil was analyzed using a Hewlett–Packard capillary GC-quadrupole MS system (Model 5972) fitted with a 25 m × 0.20 mm i.d. fused silica column coated with DB1. Temperature programme 60°C (1 min), 60–220°C at 3°C/min. Helium was used as carrier gas at a flow rate of 0.6 ml/min, the mass spectrometer was operated at 70 eV. Authentic reference compounds, as well as published mass spectra (Adams, 1989) and retention indices (Jennings & Shibamoto, 1980) were used as the basis for identification of the usual constituents.

3.4. Isolation

Chromenes I and II were obtained as two fractions A and B, with 15/85 and 80/20 ratios, respectively, by CC of the essential oil of *C. tricona* on silica gel 60 (Merck, 70–230 mesh ASTM) eluted with a pentane–Et_2O gradient. ^1H- and ^{13}C-NMR were taken at 400 MHz ($CDCl_3$) and the corresponding data are given in Table 2.

3.5. MS data

19 (chromene I), *m*/*z* (rel. int.): 191 (100), 206 (20) $[M]^+$, 176 (16), 192 (13), 205 (6).
20 (chromene II), *m*/*z* (rel. int.): 205 (100), 220 (22) $[M]^+$, 190 (17), 206 (13), 175 (11).

3.6. Synthesis

3.6.1. 3,5-Dimethoxyphenylcrotonate

Crotonylchloride (18 g, 0.2 mol) was slowly added (30 min) to a solution of 3,5-dimethoxyphenol (6 g, 0.04 mol) and *N*,*N*-dimethyl-4-aminopyridine (2.4 g, 0.02 mol) in anhydrous ethylether (10 ml). The mixture was gently heated (50°C) for 2 h and treated (after cooling) with 0.1 M aqueous sodium hydroxyde (3 × 30 ml). The organic layer, washed up to neutrality, gave, after evaporation, 3.6 g of crude ester (yield: 40%).

MS, *m*/*z* (rel. int.): 69 (100), 154 (65), 222 (35), 125 (25), 41 (25), 39 (18), 194 (10). NMR ($CDCl_3$, 400 MHz) (δ, nb H, *signal*, J): 1.90, 3H, *dd*, 1.5 and 6.9; 3.56, 3H, *s*; 3.59, 3H, *s*; 5.93, 1H, *dq*, 15.5 and 1.5; 6.21, 2H, *d*, 2.1; 6.27, 1H, *t*, 2.1; 7,10, 1H, *dq*, 15.5 and 6.9.

3.6.2. 5,7-Dimethoxy-2-methylbenzopyran-4-one

5,7-Dimethoxy-2-methylbenzopyran-4-one was obtained by Fries transposition from 3,5-dimethoxyphenylcrotonate (2.3 g) by heating with aluminium chloride (1.6 g) at 140–150°C for 2 h (Cavill, Dean, Mc Gookin, Marshall & Robertson, 1954). The cooled mixture was treated with hydrochloric acid 3% and extracted with dichloromethane. After washing of the organic layer with 0.1 M aqueous sodium hydroxide (to eliminate phenolic products), the solvent was evaporated giving an oil (1.2 g, yield: 58%); the crude product was finally purified by chromatography on a silica gel column eluted with a gradient hexane–Et_2O.

MS, *m*/*z* (rel. int.): 180 (100), 222 (72), 137 (55), 153 (53), 193 (20), 69 (18), 151 (17). NMR ($CDCl_3$, 400 MHz), (δ, nb H, *signal*, J): 1.42, 3H, *d*, 6.3; 2.65, 1H, *dd*, 16.5 and 4.6; 2.76, 1H, *dd*, 16.5 and 9.3; 3.77, 3H, *s*; 3.83, 3H, *s*; 4.48, 1H, *m*; 6.00, 1H, *d*, 2.3; 6.02, 1H, *d*, 2.3.

3.6.3. 5,7-Dimethoxy-2-methyl-2H-benzopyran (**19**)

The chromanone (1.1 g) was reduced with lithium aluminium hydride in excess in anhydrous diethylether. After acidic treatment with 10% sulfuric acid for 1 h, the organic layer was separated and gave 0.6 g of crude product which was purified by silica gel column chromatography (yield:20%, after purification). The spectral characteristics of the chromene obtained by synthesis were identical to those observed for the natural product.

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