Journal of Natural Products
Vol. 49, No. 1, pp. 95-100, Jan-Feb 1986

ADDITIONAL NEW XANTHONES AND XANTHONOLIGNOIDS FROM *HYPERICUM CANARIENSIS*

M. LUZ CARDONA, M. ISABEL FERNÁNDEZ, JOSÉ R. PEDRO, ELISEO SEOANE, and ROSA VIDAL

Department of Organic Chemistry, Faculty of Chemistry, University of Valencia, Burjassot, Valencia, Spain

ABSTRACT.—The isolation and characterization of two xanthones, a pyranoxanthone, and two xanthonolignoids from the aerial parts of *Hypericum canariensis* are reported. Two of these compounds are described for the first time.

According to Engler (1), the genus *Hypericum* has been included in the subfamily Hypericoideae of the Guttiferae, a family characterized by the occurrence of xanthones. We have previously reported several xanthone derivatives of *Hypericum ericoides* (2,3) and *Hypericum canariensis* L. (4), and we wish to report now the isolation and structure elucidation of five additional xanthone constituents. Among these compounds, a pyranoxanthone (**6**) and a xanthonolignoid (**9a**) are described for the first time, for which we propose the names of hypericanarin and cadensin D, respectively.

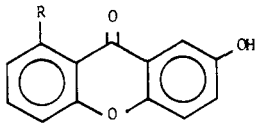
RESULTS AND DISCUSSION

Column chromatography of a $CHCl_3$ extract of the aerial parts of *H. canariensis* gave seven groups of eluates, of which the first four deposited pure compounds: 1,7-dihydroxyxanthone (**1**), 2-hydroxyxanthone (**2**), 2-hydroxy-5-methoxyxanthone (**3**), and 2,5-dihydroxyxanthone (**4**), respectively (4). By further chromatographic purification, five additional compounds **5, 6, 7, 8a,** and **9a** were obtained.

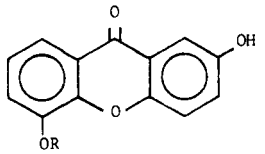
The compounds **5** and **7** were classified as xanthones on the basis of their ir and uv spectra. Their molecular weights, determined by ms, were consistent with a dihydroxydimethoxyxanthone and a hydroxydimethoxyxanthone, respectively. The ^{1}H-nmr spectrum of compound **7** confirmed the existence of two methoxyl groups and of five aromatic protons. A doublet at relatively low field (δ 8.07) was characteristic of a C-8 proton, *peri* to the carbonyl, on an unsubstituted xanthone ring (5), and a singlet at (δ 7.06) was indicative of a C-1 proton on a 2,3,4-trioxygenated ring. The hydroxyl group was situated at C-3 as it undergoes a large K-band bathochromic shift upon addition of NaOAc (6). Consequently, the methoxyl groups were placed at C-2 and C-4, which agrees with the fragments (M-CH_3) and (M-H_2CO) in the mass spectrum (7). This leads to the structure of 3-hydroxy-2,4-dimethoxyxanthone for compound **7**. This compound has been reported previously from two *Kielmeyera* species (8-10) [subfamily Kielmeyeroideae, family Guttiferae (1)], but this is the first time that it has been isolated from the subfamily Hypericoideae.

The ^{1}H-nmr spectrum of compound **5** confirmed the existence of two methoxyl groups and of four aromatic protons. These protons give typical signals of three vicinal protons and a singlet at δ 7.13 indicative of a proton, *peri* to the carbonyl, on a trioxygenated ring. The hydroxyl groups were situated at C-1 and C-6, as the uv maxima in MeOH shows a bathochromic shift upon adding $AlCl_3$ and NaOAc (6). The methoxyl groups were situated at C-2 and C-4 since the molecular ion undergoes the loss of methyl and H_2CO groups (7). Consequently, the structure of 1,6-dihydroxy-5,7-dimethoxyxanthone was assigned to compound **5**. This compound has been reported only once (11) from *Caraipa densiflora* subfamily Kielmeyeroideae (1), but this is the first report of it in the subfamily Hypericoideae.

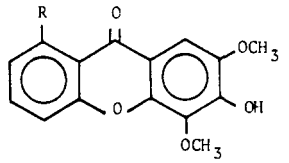
Compound **6** was a new natural product, for which we propose the name of hypericanarin. The relative intensity of isotopic molecular ions gave the molecular for-



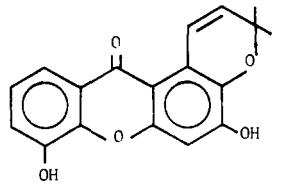
1 R=OH
2 R=H



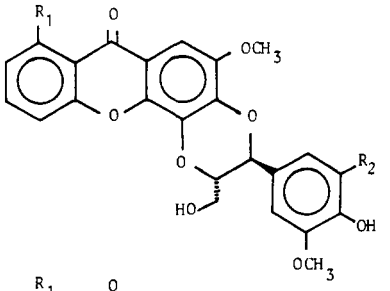
3 $R=CH_3$
4 R=H



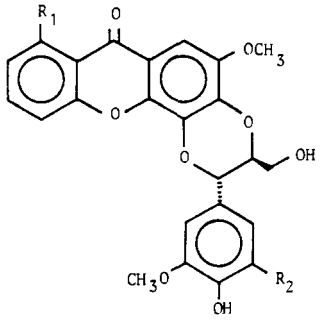
5 R=OH
7 R=H



6



8a $R_1=R_2=H$
9a $R_1=H$, $R_2=OCH_3$
10a $R_1=OH$, $R_2=OCH_3$



8b $R_1=R_2=H$
9b $R_1=H$, $R_2=OCH_3$
10b $R_1=OH$, $R_2=OCH_3$

mula $C_{18}H_{14}O_5$. The uv spectrum of hypericanarin was suggestive of a xanthone. The molecular carbon content is thus compatible with a xanthone nucleus substituted with one C_5-unit. The ^1H nmr of hypericanarin showed a pair of doublets at δ 7.96 and 5.68 ($J=10.2$ Hz) as well as a six-proton singlet at δ 1.37, which were indicative of a 2,2-dimethyl-2H-pyran ring. The characteristic downfield shift of one of the vinylic proton doublets (δ 7.96) of the chromene ring strongly suggested (12) the chromene ring to be angularly cyclized and also adjacent to the xanthone carbonyl group. The angular position of the pyran ring was further suggested by the absence of any uv absorption band in the region 280-290 nm, in contrast to those xanthones with a linearly cyclized pyran ring (13,14). To complete the molecular formula of hypericanarin, two hydroxyl groups were needed. The uv spectrum in MeOH showed a large K-band bathochromic shift on addition of NaOAc, indicating the *para*-relationship of one hydroxyl and the carbonyl group (6). Consequently, this hydroxyl group must be situated on the same ring as the pyran ring (C-3) because the aromatic region of the ^1H-nmr spectrum contains typical signals of three vicinal protons. The second hydroxyl group must be placed on C-4 of the other ring as its uv spectra in MeOH and in (MeOH + $AlCl_3$) are superim-

posable. Thus, the structure of hypericanarin was established as **6**. This is the first report on this pyranoxanthone.

The compound **8a**, on the basis of spectroscopic evidence, was identified as the xanthonolignoid kielcorin, previously isolated from different species of *Kielmeyera* (15) and *Hypericum* (3,16).

The compound **9a** showed a molecular ion at *m/z* 466 in agreement with a molecular formula $C_{25}H_{23}O_9$. A xanthone nucleus was evident from the ir (ν max 1640, 1610, 1220, 1140 cm^{-1}) and uv (242, 253, 317, and 363 nm) spectra. The ^1H nmr showed a double doublet at relatively low field (δ 8.21), which is indicative of a proton *peri* to the carbonyl group (C-8) on an unsubstituted xanthone ring (5). Furthermore, the value of the coupling constants (J=8.1 and 1.7 Hz) indicated the existence of protons on C-7 and C-6. The proton on C-7 appeared (δ 7.48) as a triple doublet (J=7.9 and 0.6 Hz), and the proton on C-6 appeared (δ 7.86) as a triple doublet also (J=7.9 and 1.0 Hz) which indicated the existence of the proton on C-5. The signal corresponding to this proton appeared at δ 7.69 as a double doublet (J=8.1 and 0.6 Hz). The ^1H-nmr spectrum also showed a singlet at δ 7.22, characteristic of a proton at C-1 on a 2,3,4-trioxygenated xanthone. Three methoxyl groups were also observed in the ^1H-nmr spectrum. Subtraction of the xanthone nucleus (13 carbon atoms) and the three methoxyl groups from the C-25 framework left another C-9 unit to be accounted for. On biogenetic grounds and by similarity with kielcorin **8a**, it was considered that the C-9 unit was a C_6-C_3 phenylpropane moiety. As in kielcorin, the observation of a deshielded doublet (δ 5.07) typical for a benzylic methyne substituted by oxygen, and its typical *trans*-coupling (J=8.0 Hz) seemed to indicate the existence of a *trans*-substituted 1,4-dioxane ring between the xanthone framework and the phenyl ring. In support of this, the base peak at *m/z* 210 could be rationalized in terms of a retro-Diels Alder reaction in the dioxane ring. The ions at *m/z* 210, 192, 182, 181, 167, and 154 (which appeared at *m/z* 180, 162, 152, 151, 137, and 124, respectively, in kielcorin) indicated that one phenolic group and two methoxyl groups were present on the phenyl ring. From a comparison with alternative substitution patterns, the multiplicity (a singlet) and the chemical shift of the two remaining aromatic protons indicated a 4-hydroxy-3,5-dimethoxy-substituted phenyl ring. The remaining methoxy group was situated at C-2 (xanthone framework) because the framework at *m/z* 258 corresponding

TABLE 1. ^1H-nmr Chemical Shifts of Xanthonolignoids, Kielcorin (**8a**), and Cadensin D (**9a**) in DMSO-d_6[a]

	Compound **8a**	Compound **9a**
H-8	8.18, dd, J=8.1 and 1.7 Hz	8.21, dd, J=8.1 and 1.7 Hz
H-6	7.82, td, J=7.1	7.86, td, J=7.9 and 1.0 Hz
H-5	7.63, dd, J=8.1	7.69, dd, J=8.1 and 0.6 Hz)
H-7	7.44, td, J=7.1	7.48, td, J=7.9 and 0.6 Hz
H-1	7.19, s	7.22, s
H-2″	7.07, br.s	6.78 } s
H-6″	6.87 } br.s	6.78 }
H-5″	6.87 }	
H-6′	5.07, d, J=7.9 Hz	5.07, d, J=8.0 Hz
H-5′	4.36 m	4.45 m
MeO-2	3.84 s	3.87 s
MeO-3″	3.79 s	3.77 } s
MeO-5″		3.77 }

[a]The spectra of **8a** and **9a** have been measured at 90 and 200 MHz, respectively. All chemical shift values are given in δ (ppm) relative to TMS.

TABLE 2. Mass Spectral Fragmentation Patterns of Xanthonolignoids, Kielcorin (**8a**), Cadensin D (**9a**), and Cadensin C (**10a**)

Ion	Compound **8a**	Compound **9a**	Compound **10a**
	m/z (%)	*m/z* (%)	*m/z* (%)
M^+	436 (20.3)	466 (6.9)	482 (2.4)
M^+-H_2O	418 (2.2)	448 (0.9)	—
M^+-$ArCH_2$	299 (5.0)	299 (2.6)	315 (0.9)
M^+-$ArC\equiv CCH_2OH{=}A^+$	258 (38.6)	258 (25.4)	274 (12.8)
A^+-CH_3	243 (23.6)	243 (16.6)	259 (9.7)
A^+-H_2CO	228 (10.7)	228 (11.4)	244 (5.7)
A^+-CH_3-H_2O	225 (4.2)	225 (3.4)	241 (1.8)
A^+-CH_3-CO	215 (14.6)	215 (10.2)	231 (3.7)
A^+-CH_3-2CO	187 (11.0)	187 (10.2)	203 (5.2)
$ArCH{=}CH\text{-}CH_2OH{=}B^+$	180 (73.2)	210 (100)	
$ArC\equiv CCH_2OH$	178 (2.2)	208 (11.8)	
B^+-H_2O	162 (15.7)	192 (6.3)	
B^+-CO	152 (13.5)	182 (52.9)	
B^+-HCO	151 (8.7)	181 (19.8)	
$ArCH_2$	137 (100)	167 (7.20)	
ArH	124 (71.3)	154 (40.8)	
$ArCH_2$-H_2O	119 (27.0)	149 (32.5)	
$ArCH_2$-H_2O-CO	91 (47.7)	121 (24.0)	

to the xanthone framework underwent the loss of the methyl group (followed by two successive losses of CO) and the loss of CH_2O (7). All of these spectroscopic properties led to the proposition of two alternative structures **9a** or **9b**. Of these two possible structures, we have chosen the former, according to the result obtained by ethanolysis (16). The treatment with EtONa led to the opening of the dioxane ring; the resultant phenolic group was situated at C-3 of the xanthone nucleus, as indicated by a large K-band bathochromic shift in the uv spectrum, by addition of NaOAc (6). Because the optical activity of compound **9a** was zero, $[\alpha]^{20}D=0°$, and the relative configuration was *trans*, the natural product must include both the *5R, 6R* and *5S, 6S* enantiomers. This compound is described here for the first time, and we propose the name of cadensin D. The mass spectrum of cadensin D (**9a**) displayed a small peak at $M^+ + 16$, and its uv spectrum showed a widening of the band at 319 nm upon addition of $AlCl_3$, which indicated that the compound **9a** presumably contains a small amount of its 8-hydroxyderivative **10a**, named cadensin C (17). This compound has been isolated as an acetyl derivative only once from *Vismia guaramirangae* (17).

Xanthonolignoids are a relatively rare group of natural products. Gottlieb and co-workers were the first to isolate three xanthonolignoids, kielcorin from *Kielmeyera* species (15) and cadensins A and B from *Caraipa densiflora* (15), but they were unable to distinguish between the two alternative structural isomers **8a** and **8b**. In further work, Nielsen and Arends (16) isolated kielcorin again from *Hypericum* species and demonstrated the structure **8a**. Delle Monache and co-workers isolated the fourth xanthonolignoid, cadensin C, **10a** from *Vismia guaramirangae* (17), and in this paper, we report the isolation of the fifth, cadensin D, **9a** from *H. canariensis*.

Biogenetically, kielcorin **8a** and cadensin D **9a** may be derived from 3,4-dihydroxy-2-methoxyxanthone through a radical coupling process with coniferyl alcohol and syringenin, respectively, such as is postulated for the biosynthesis of flavonolignoids (18) and coumarinolignoids (19).

Finally, it is interesting to note that the compounds we have isolated establish a

link between the subfamilies Kielmeyeroideae and Hypericoideae, of the family Guttiferae, according to Engler (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot plate and are uncorrected. Spectra were recorded with the following instruments: ms, Varian 160 (70 ev); ^{1}H nmr, Varian XL-200 (200 MHz); ir, Perkin-Elmer 281, and uv, Perkin-Elmer 575.

EXTRACTION AND FRACTIONATION.—*H. canariensis* was collected and classified as described by Cardona *et al.* (4). We obtained from aerial parts (3.5 kg) a $CHCl_3$ extract (25 g) which was chromatographed on silica gel (310 g). Hexane/EtOAc of increasing polarity eluted 250 fractions, combined into seven groups, as Table 3 shows. Compounds **1, 2, 3,** and **4** crystallized directly from the eluate, and they were identified as 1,7-dihydroxyxanthone (**1**), 2-hydroxyxanthone (**2**), 2-hydroxy-5-methoxyxanthone (**3**), and 2,5-dihydroxyxanthone (**4**), respectively, as described in the first report (4). The remaining compounds were obtained after further chromatographic purifications.

TABLE 3. Compounds Isolated from $CHCl_3$ Extract of *H. canariensis*

Eluates	Fractions	Proportion of Hexane-EtOAc	Compounds
I	54-58	85:15	**1**
II	72-78	80:20	**2**
III	84-96	80:20	**3,5,6**
IV	109-123	75:25	**4**
V	124-144	70:30	**7**
VI	192-206	40:60	**8a**
VII	207-242	40:60	**8a,9a** (and **10a**)

1,6-DIHYDROXY-5,7-DIMETHOXYXANTHONE.—Compound **5** was obtained (7 mg) from the third group of eluates by preparative tlc ($CHCl_3$-EtOAc, 7:3); mp 231-233° (from $CHCl_3$/EtOAc) [lit (11) mp 229-231°]; ms *m/z* (rel. int.) 288 (100, M^+), 273 (19.8, M-CH_3), 258 (10.8, M-H_2CO), 244 (15.6), 242 (4.5), 229 (13.3), 214 (4.2); uv λ max (MeOH) nm 219 (log ε 4.19), 240sh (4.22), 255 (4.40), 313 (3.93), and 373 (3.68); λ max (MeOH+NaOMe) nm 248, 268, 280sh, 350, and 408sh; λ max (MeOH+NaOAc) nm 248, 268, 280sh, 345, and 388sh; λ max (MeOH+$AlCl_3$) nm 212, 234, 269, 290, 335, 350sh, and 438, which do not change by addition of HCl; ir (KBr) cm^{-1} 3550-3100, 2930, 2850, 1645, 1618, 1580, 1500, 1455, 1440, 1400, 1230, 1130, 1100, 1070, 797, 730, and 698; ^{1}H nmr (DMSO-d_6) δ 10.18 (s, OH), 7.73 (t, J=8.4 Hz, H-3), 7.13 (s, H-8), 7.10 (dd, J=8.5 and 0.9 Hz, H-4), 6.77 (dd, J=8.2 and 0.9 Hz, H-2), 3.91 and 3.87 (2s, $2CH_3O$-).

HYPERICANARIN.—Compound **6** was obtained (45 mg) from the third group of eluates by preparative tlc ($CHCl_3$-EtOAc, 7:3). It darkened from 240°, but it did not show a defined melting point; ms *m/z* (rel. int.) 310 (M^+, 31.3), 309 (12.5), 295 (100, M-CH_3), 267 (3.7); uv λ max (MeOH) nm 240 (log ε 4.08), 260 (3.97), 306 (3.71), and 382 (3.44); λ max (MeOH+NaOMe) nm 260, 268sh, 296, 343, and 380; λ max (MeOH+NaOAc) nm 229, 239, 300, 315, and 375; ir (KBr) cm^{-1} 3500-3100, 2920, 2845, 1560, 1450, 1387, 1340, 1290, 1130, 960, 904, 836, 812, 782, and 754; ^{1}H nmr (DMSO-d_6) δ 7.96 (d, J=10.2 Hz, Ar-C*H*=C), 7.43 (dd, J=7.6 and 1.7 Hz, H-8), 7.15 (dd, J=7.7 and 1.7 Hz, H-6), 7.04 (t, J=7.6 Hz, H-7), 6.4 (s, H-4), 5.68 (d, J=10.2 Hz, C=C*H*-), and 1.37 (s, $2CH_3$-).

3-HYDROXY-2,4-DIMETHOXYXANTHONE.—Compound **7** was obtained (14 mg) from the fifth group of eluates by column chromatography and preparative tlc ($CHCl_3$-EtOAc, 8:2); mp 222-224° (from hexane/EtOAc), [lit (8,9) mp 224-226°]; ms *m/z* (rel. int.) 272 (100, M^+), 257 (39.0), M-CH_3), 242 (3.6, M-H_2CO), 227 (4.1), 214 (15.4), 211 (15.7), 186 (5.0), and 171 (3.4); uv λ max (MeOH) nm 239 (log ε 4.47), 280sh (3.84), 316 (4.04), and 355sh (3.95); λ max (MeOH+NaOMe) nm 232, 275, and 383; λ max (MeOH+NaOAc) are the same as in (MeOH+NaOMe); ir (KBr) cm^{-1} 3550-3050, 2920, 2855, 1600, 1465, 1430, 1383, 1332, 1225, 1135, 1090, 880, and 755; ^{1}H nmr (DMSO-d_6) δ 8.07 (dd, J=7.9 and 1.7 Hz, H-8), 7.63 (td, J=8.0 and 1.5 Hz, H-6), 7.48 (dd, J=8.3 and 0.9 Hz, H-5), 7.31 (dt, J=7.5 and 0.9 Hz, H-7), 7.06 (s, H-1), 3.79 and 3.74 (2s, $2CH_3O$-).

KIELCORIN.—Compound **8a** was obtained (20 mg) from the sixth group of eluates by column chromatography and from the seventh group by preparative tlc ($CHCl_3$-EtOAc, 1:1, three elutions), mp 252-253° (from MeOH) [lit (15), mp 250-251°]; $[\alpha]^{20}D$=0°; uv λ max (MeOH) nm 239 (log ε 4.29), 253

(4.25), 287 (3.72), 318 (3.85), and 362sh (3.44); λ max (MeOH+NaOMe) nm 257, 290, 319, and 362sh; ir (KBr) cm^{-1} 3600-3100, 3080, 3020, 2940, 2860, 1635, 1610, 1520, 1485, 1465, 1400, 1355, 1280, 1230, 1220, 1140, 1110, 1070, 1055, 895, 870, 840, 815, 755, 700, and 635.

CADENSIN D.—Compound **9a** was obtained (8 mg) from the seventh group of eluates by preparative tlc ($CHCl_3$-EtOAc, 1:1, three elutions); mp 243-245° (from MeOH); $[\alpha]^{20}D=0°$; uv λ max (MeOH) nm 242 (log ε 4.51), 253 (4.50), 317 (4.18), and 363sh (3.77); λ max (MeOH+NaOMe) nm 255, 275sh, 319, and 360sh; ir (KBr) cm^{-1} 3600-3050, 2920, 2850, 1640, 1610, 1482, 1465, 1400, 1325, 1220, 1140, 1110, 810, 755, 700, 672, and 640.

ETHANOLYSIS PRODUCT.—Cadensin D (1 mg) in 1 ml of EtONa/EtOH (1.2N) was heated at reflux for 0.5 h, then acidified with diluted HCl, extracted with $CHCl_3$, and separated by preparative tlc ($CHCl_3$-EtOAc, 7:3, two elutions) on silica gel. Uv λ max (MeOH) 236, 270sh, 315, and 350sh; λ max (MeOH+NaOAc), 223, 280sh, and 376.

ACKNOWLEDGMENTS

The authors wish to thank Prof. Pérez de Paz, Department of Botany, University of La Laguna, Tenerife, for the collection and classification of the plant material and Prof. Castells and Dr. Feliz, Department of Organic Chemistry, University of Barcelona, for recording several ^1H-nmr spectra.

LITERATURE CITED

1. M. Melchoir, *A Engler's Syllabus der Pflanzenfamilien*, 12th ed., Vol. 2, Gebrüder Bornträger, Berlin, 1964, p. 444.
2. M.L. Cardona and E. Seoane, *J. Nat. Prod.*, **45**, 134 (1982).
3. M.L. Cardona and E. Seoane, *Phytochemistry*, **21**, 2759 (1982).
4. M.L. Cardona, J.R. Pedro, E. Seoane, and R. Vidal, *J. Nat. Prod.*, **48**, 467 (1985).
5. D. Barrachlongh, H.D. Locksley, F. Scheinmann, M.T. Magalhaes, and O.R. Gottlieb, *J. Chem. Soc.*, **B**, 603 (1970).
6. A.A.L. Mesquita, D.B. Correa, O.R. Gottlieb, and M.T. Magalhaes, *Anal. Chim. Acta*, **42**, 311 (1968).
7. P. Arends, P. Helboe, and J. Moller, *Org. Mass. Spectrom.*, **7**, 667 (1973).
8. G.G. de Oliveira, A.A.L. Mesquita, O.R. Gottlieb, and M.T. Magalhaes, *An. Acad. Brasil. Cienc.*, **40**, 29 (1964).
9. O.R. Gottlieb, A.A.L. Mesquita, G.G. de Oliveira, and M. Teixeira de Melo, *Phytochemistry*, **9**, 2537 (1970).
10. O.R. Gottlieb, A.A.L. Mesquita, and T.J. Nagem, *Phytochemistry*, **10**, 2253 (1971).
11. R. Alves de Lima, O.R. Gottlieb, and A.A.L. Mesquita, *An. Acad. Brasil. Cienc.*, **42**, 133 (1970).
12. S.J. Gabriel and O.R. Gottlieb, *Phytochemistry*, **11**, 3035 (1972).
13. A.K. Sen, K.K. Sarkar, P.C. Mazumder, N. Banerji, R. Unsvuori, and T.A. Hase, *Phytochemistry*, **19**, 2223 (1980).
14. A.K. Sen, K.K. Sarkar, P.C. Mazumder, N. Banerji, R. Unsvuori, and T.A. Hase, *Phytochemistry*, **21**, 1747 (1982).
15. J.F. Castelão, O.R. Gottlieb, R.A. Lima, A.A. Mesquita, H.E. Gottlieb, and E. Wenkert, *Phytochemistry*, **16**, 735 (1977).
16. H. Nielsen and P. Arends, *Phytochemistry*, **17**, 2040 (1978).
17. F. Delle Monache, M. Marquina Mac-Quhae, G. Delle Monache, G.B. Marini Bettolo, and R. Alves de Lima, *Phytochemistry*, **22**, 227 (1983).
18. L. Merlini, A. Zanarotti, A. Pelter, M.P. Rochefort, and R. Hänsel, *J. Chem. Soc., Perkin Trans. 1.*, 775 (1980).
19. M. Arisawa, S.S. Handa, D.D. McPherson, D.C. Lankin, G.A. Cordell, H.H.S. Fong, and N.R. Farnsworth, *J. Nat. Prod.*, **47**, 300 (1984).

Received 20 May 1985