Pergamon

PII: S0031-9422(96)00284-1

Phytochemistry, Vol. 43, No. 2, pp. 513–520, 1996

0031-9422/96 $15.00 + 0.00

XANTHONES FROM *HYPERICUM ROEPERANUM*

GUIDO RATH, OLIVIER POTTERAT, STEPHEN MAVI* and KURT HOSTETTMANN†

Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015, Lausanne, Switzerland; *National Herbarium and National Botanic Garden, P.O. Box 8100, Causeway, Zimbabwe

(*Received in revised form* 27 *March* 1996)

Key Word Index—*Hypericum roeperanum*; Guttiferae; xanthones; 1,6-dihydroxy-5-methoxy-4′,5′-dihydro-4′,4′,5′-trimethylfurano-(2′,3′:3,4)-xanthone; 1,6-dihydroxy-5-methoxy-6′,6′dimethylpyrano-(2′,3′:3,4)-xanthone; 1,3,5,6-tetrahydroxy-2-(2′,2′-dimethyl-4′-isopropenyl)-cyclopentanyl-xanthone; 1,3,5,6-tetrahydroxy-4-*trans*-sesquilavandulylxanthone; roeperanone; antifungal activity.

Abstract—Four new xanthones have been isolated from the roots of *Hypericum roeperanum*. Their structures have been established by a combination of spectroscopic and chemical methods as 1,6-dihydroxy-5-methoxy-4′,5′-dihydro-4′,4′,5′-trimethylfurano-(2′,3′:3,4)-xanthone (5-*O*-methyl-2-deprenylrheediaxanthone B), 1,6-dihydroxy-5-methoxy-6′,6′-dimethylpyrano-(2′,3′:3,4)-xanthone (5-*O*-methylisojacareubin), 1,3,5,6-tetrahydroxy-2-(2′,2′-dimethyl-4′-isopropenyl)-cyclopentanylxanthone (5-*O*-demethylpaxanthonin) and 1,3,5,6-tetrahydroxy-4-*trans*-sesquilavandulylxanthone (roeperanone). In addition, 2-hydroxyxanthone, 5-hydroxy-2-methoxyxanthone, 1,5-dihydroxy-2-methoxyxanthone, 2-deprenyl rheediaxanthone B, isojacareubin and calycinoxanthone D have been isolated and characterized. Some of the isolated xanthones exhibited antifungal activity against *Candida albicans*.

INTRODUCTION

There has been a recent increasing interest in the genus *Hypericum*, because it is a source of a variety of compounds with different biological activities. In particular, extracts of *H. perforatum* are now widely used in Europe as drugs for the treatment of depression.

In this context, we report now on the xanthones of *H. roeperanum* Schimp. ex A. Rich. [1]. *H. roeperanum* is a shrub or small tree growing in central, eastern and south tropical Africa. It is employed, alone or in association with various plants or animal parts, to cure female sterility [2] but nothing is known about its constituents.

Xanthones are widespread within the Guttiferae [3] and have been reported to possess several biological properties, including cytotoxic, antitumour, mutagenic, antimicrobial and anti-inflammatory activities [4]. Recently, increasing attention has been given to these polyphenols because of their inhibitory effect on monoamine oxidases (MAO) [5,6], and their possible potential as new antidepressant drugs.

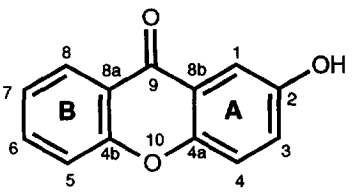
RESULTS AND DISCUSSION

From the dichloromethane extract of the roots of *H. roeperanum*, 10 xanthones have been isolated (see Experimental). They include simply oxygenated xanthones (**1–3**) and more complex prenylated derivatives (**4–10**).

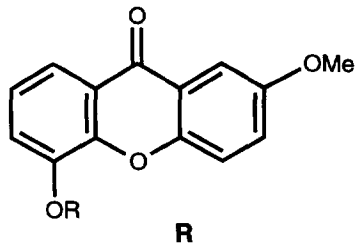
Compounds **1** and **2** were identified as 2-hydroxyxanthone [7, 8] and 5-hydroxy-2-methoxyxanthone [9], respectively, from their spectroscopic data (^{1}H and ^{13}C NMR, mass, and UV). Because of some inconsistency of the ^{1}H NMR data of compound **2** with published values [9], identification of this compound was confirmed by characterization of the methylated derivative **2a**. The ^{1}H NMR data of compound **2a** were in perfect agreement with data reported for 2,5-dimethoxyxanthone [10]. Compounds **1** and **2** have been previously isolated from *H. inodorum* [10].

The EI-mass spectrum of compound **3** showed a $[M]^+$ at *m/z* 258, corresponding to the molecular formula $C_{14}H_{10}O_5$. Due to the loss of methyl, hydroxyl or methoxyl substituents, fragments were detected at *m/z* 243, 241 and 228, respectively. The UV spectrum exhibited maxima at 206, 257, 320 and 374 nm and was similar to the spectrum of 1,5-dihydroxyxanthone [5]. Addition of NaOMe generated shifted maxima at 215, 255, 355 nm, together with an extended shoulder around 425 nm. The UV spectrum in NaOAc solution revealed features typical of a 1,5-dihydroxyxanthone [11]. Upon addition of $AlCl_3$, reduction of intensity and a bathochromic shift of 18 nm were observed for the

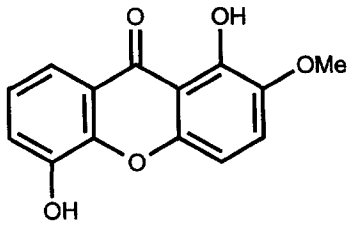
†Author to whom correspondence should be addressed.



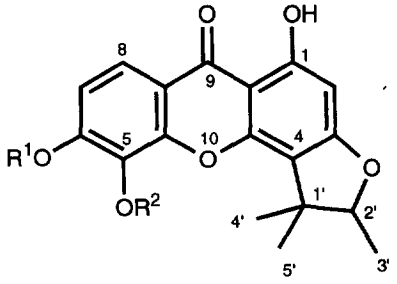
1



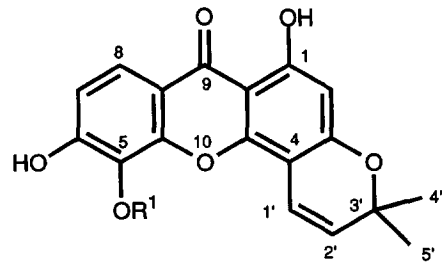
	R
2	H
2a	Me



3



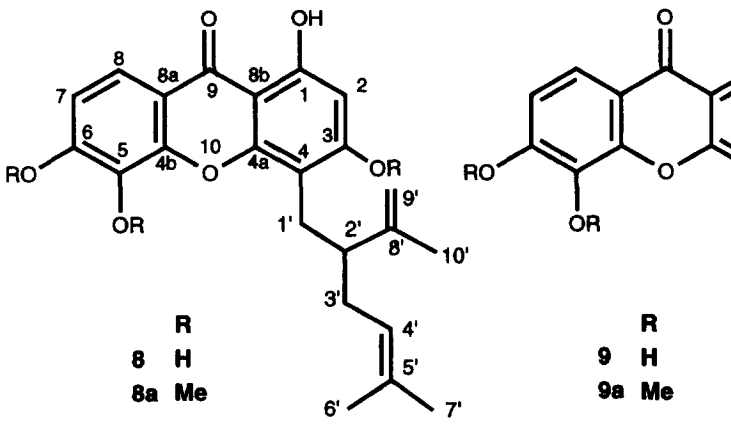
	R^1	R^2
4	H	H
4a	Me	Me
6	H	Me



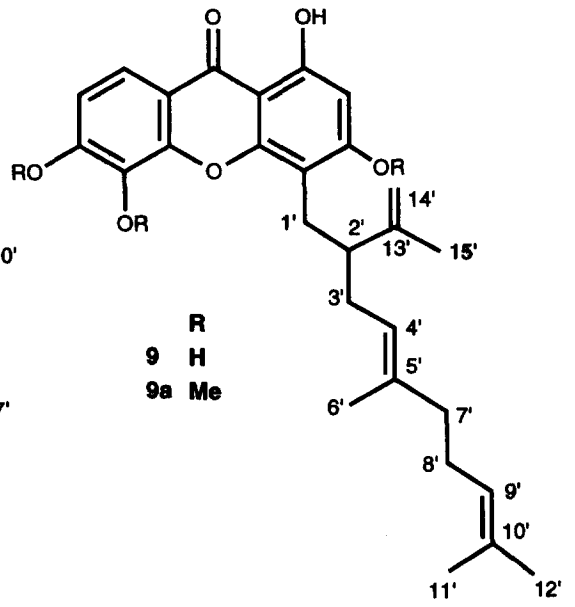
	R^1
5	H
7	Me

second principal maximum (λ 274 nm) which was indicative of a chelated hydroxyl group [11]. This was further confirmed by a broad singlet at δ 12.70 in the ^{1}H NMR spectrum. In addition, the ^{1}H NMR spectrum of compound **3** showed a singlet at δ 3.86, characteristic of an aromatic methoxyl group and two aromatic protons as an AB system at δ 7.58 and δ 7.09 (2*d*, $J = 9.2$ Hz), revealing a 1,2,3,4-substituted benzene ring. NOE difference measurements established the position of the methoxyl group and also the correct attribution of H-3 and H-4. Thus, upon irradiation of the methoxyl substituent, enhancement of the doublet at δ 7.58 (H-3) was observed. The chemical shifts of the B-ring protons observed as an ABM system at δ 7.27 (*t*, $J = 7.8$ Hz), 7.36 (*dd*, $J = 7.8$, 1.9 Hz) and 7.61 (*dd*, $J = 7.8$, 1.9 Hz) were almost identical to those of 1,5-dihydroxyxanthone [5]. This demonstrated the second hydroxyl group to be at C-5. Thus, compound **3** is 1,5-dihydroxy-2-methoxyxanthone. This compound has been previously isolated from *Garcinia xanthochymus* [12], but no spectroscopic data have been reported.

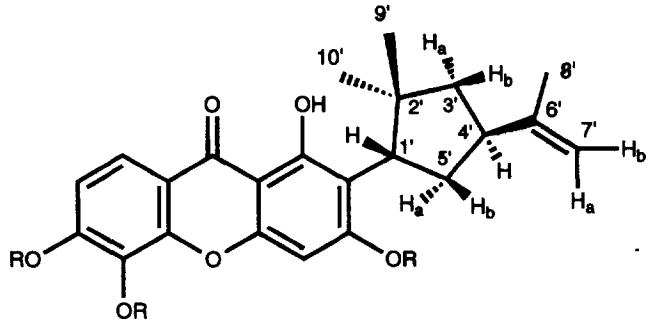
Compound **4** gave a $[M]^+$ at *m/z* 328 by EI-mass spectrometry. The UV spectrum was characteristic of a 1,3,5,6-tetraoxygenated xanthone lacking additional conjugation [13]. ^{13}C NMR data showed the molecular formula to be $C_{18}H_{16}O_6$. The ^{1}H NMR spectrum exhibited two doublets at δ 7.58 and δ 6.95 (2*d*, $J =$ 8.7 Hz, H-7, H-8), a singlet at δ 6.10 (H-2) and a chelated hydroxyl at δ 13.43. The signals in the high-field region of the ^{1}H NMR spectrum showed the A-ring of compound **4** to be substituted by a 2,3-dihydro-2,3,3-trimethylfuran moiety (δ 4.53, *q*, $J =$ 6.6 Hz, H-2'; δ 1.59, 1.29, gem-dimethyl; δ 1.36, *d*, $J = 6.6$ Hz, H_3-3'). When the spectrum was measured in pyridine-d_5, the signal of the proton at δ 6.10 (H-2) underwent a large paramagnetic shift (+0.42 ppm).



R
8 H
8a Me



R
9 H
9a Me



R
10 H
10a Me

Therefore the 2,3-dihydro-2,3,3-trimethylfuran ring must be closed at C-4 [14]. Further evidence for the angular arrangement of the furan ring was supplied by a negative Gibbs reaction [15] of compound **4a** prepared by methylation of **4** with diazomethane. ^{1}H NMR, ^{13}C NMR and EI-mass spectral data of compound **4** were in good agreement with data given for 2-deprenyl-rheediaxanthone B [16], obtained as a degradation product of rheediaxanthone B. However, while 2-deprenyl-rheediaxanthone B was reported to be optically active [16], compound **4** lacks optical activity. Therefore it cannot be excluded that the furan ring of **4** was formed by cyclization of a α,α-dimethylallyl chain during the drying of the plant material or the isolation procedure. 2-Deprenyl-rheediaxanthone B (**4**) has been obtained, to our knowledge, for the first time from a natural source.

Compound **5** gave a $[M]^+$ peak at m/z 326 in the EI-mass spectrum. The UV spectrum was characteristic of a 1,3,5,6-tetraoxygenated xanthone. A distinct shoulder at 365 nm [13] revealed conjugation with a pyran ring. EI-mass spectra, ^{1}H and ^{13}C NMR data (Table 1) were in complete agreement with those of isojacareubin, previously isolated from *H. japonicum* [17]. This is only the second report of the isolation of isojacareubin from a plant source.

The molecular formula of compound **6** was deduced to be $C_{19}H_{18}O_6$ from the EI-mass spectrum ($[M]^+$ at m/z 342) and ^{13}C NMR data. The UV spectrum of compound **6** exhibited maxima at 241, 314 and 356 nm. $AlCl_3$ induced a bathochromic shift (44 nm), unchanged after addition of HCl. A bathochromic shift of 18 nm caused by sodium acetate was indicative for a hydroxyl at C-3 or C-6. The ^{1}H NMR spectrum revealed the presence of a chelated hydroxyl (δ 13.40), three aromatic protons, appearing as two doublets at δ 7.85 and 7.03 (2*d*, $J = 8.6$ Hz, H-7, H-8) and a singlet at δ 6.18 (H-2). Further, it showed the presence of a 2,3-dihydro-2,3,3-trimethylfuran ring (δ 4.60, *q*, $J = 6.6$ Hz, H-2′; δ 1.43, *d*, $J = 6.6$ Hz, H_3-3′; δ 1.65, 1.35, gem-dimethyl) and a methoxyl group (δ 4.02) which proved to be di-ortho substituted, because it was observed at δ 61.8 in the ^{13}C NMR spectrum [18]. Absence of signal enhancement upon irradiation of the

Table 1. ^{13}C NMR spectral data of compounds **2**, **4** and **5**–**10** in acetone-d_6, except for **2** which was measured in DMSO-d_6

C	2	4	5	6	7*	8	9	10
1	105.7	166.8[a]	164.2	166.9[a]		163.7	163.7	164.8
2	155.5	93.8	99.4	92.8	99.6	98.2	98.2	111.3
3	123.7[a]	165.2[a]	161.2	165.2[a]		162.4	162.4	163.6
4	119.7	113.4	102.1	113.8		114.7	114.7	94.5
4a	150.0	152.6	153.0[a]	153.6		156.3	156.3	156.5
4b	145.1	147.1	147.0	151.4		147.3	147.3	146.8
5	146.5	133.7	133.5	135.7		133.5	135.4[a]	133.3
6	115.0	152.6	152.7[a]	157.5		152.2	152.3	152.2
7	124.4[a]	113.4	113.9	114.5	114.8	113.3	113.4	113.7
8	119.7	117.5	117.5	122.3	122.1	117.4	117.4	117.4
8a	121.2[b]	114.9	114.6	114.9		107.1	107.1	114.8
9	175.8	181.1	181.4	180.7		181.5	181.5	181.3
8b	121.5[b]	103.5	103.5	103.6		103.0	103.0	102.9
1′		44.5	115.9	44.5	115.3	27.7	27.7	44.2
2′		91.6	128.0	91.6	128.6	47.8	47.9	45.8
3′		21.4	78.9	21.7	79.0	32.2	32.1	48.3
4′		25.8	28.3	25.8	28.4	124.4	125.2[b]	44.9
5′		14.5	28.3	14.4	28.4	131.7	133.6[a]	33.3
6′						17.9	16.2	150.3
7′						25.8	40.4	108.3
8′						148.9	27.3	21.2
9′						111.5	124.4[b]	30.5
10′						19.0	131.4	25.1
11′							17.7	
12′							25.8	
13′							149.0	
14′							111.4	
15′							19.0	
OMe	55.6			61.8	61.7			

Assignments by comparison with data reported for related compounds.

*Due to scarcity of sample, only a part of signals were assigned.

[a,b]Assignments with the same superscripts in each column are interchangeable.

methoxyl group brought additional evidence for allocation of the methoxyl group at C-5 and a hydroxyl substituent at C-6. Measurement of the ^1H NMR spectrum in pyridine-d_5 caused a large paramagnetic shift of the singlet at δ 6.18 (+0.36 ppm) [14]. The pronounced paramagnetic shift in compound **6** and a negative Gibbs reaction [15] of the methylated derivative, which was identical to compound **4a**, permitted the assignment of an angular trimethylfuran structure. Interestingly, in contrast to 2-deprenylrheediaxanthone B (**4**), 1,6-dihydroxy-5-methoxy-4′,5′-dihydro-4′,4′,5′-trimethylfurano-(2′,3′:3,4)-xanthone (**6**) which is the 5-methyl ether derivative of compound **4**, is optically active. Xanthone **6** is a new natural compound.

Compound **7** gave a $[M]^+$ peak at m/z 340 in the EI-mass spectrum. The UV spectrum was characteristic of a 1,3,5,6-tetraoxygenated xanthone with an additional conjugated double bond [13]. The mass difference of 2 amu compared with **6**, and also the change in the UV spectrum suggested a similar structural relation between compounds **6** and **7** as to that between compounds **4** and **5**. The molecular formula was found to be $C_{19}H_{16}O_6$. The UV spectrum of compound **7** exhibited maxima at 251, 319 and 367 nm. $AlCl_3$ caused a pronounced bathochromic shift (49 nm), unchanged after addition of HCl. Addition of sodium acetate strongly increased the intensity of the UV spectrum and caused a small bathochromic shift (7 nm). The ^1H NMR spectrum of compound **7** confirmed the presence of a chelated hydroxyl (δ 13.22) and revealed a 2,2-dimethyl pyran ring (δ 6.90, *d*, J = 10.1 Hz, H-1′; δ 5.81, *d*, J = 10.1 Hz, H-2′; δ 1.49, *s*, H_3-4′ and H_3-5′). It showed further two aromatic ortho coupled protons at δ 7.84 and δ 7.05 (2*d*, J = 9.0 Hz, H-7, H-8) and an aromatic methoxyl substituent (δ 4.05). Attachment of the latter to C-5 was clearly indicated by its low-field resonance in the ^{13}C NMR spectrum (δ 61.7), due to di-ortho substitution [18]. Similar to the angular constituent isojacareubin (**5**), the high-field singlet in the spectrum of compound **7** at δ 6.18 underwent a large paramagnetic shift in pyridine-d_5 (+0.36 ppm) while the chemical shift difference ($\Delta\delta = \delta$ (pyridine-d_5)-δ (acetone-d_6)) for the α CH (H-1′) of the pyran ring was found to be negative (−0.12 ppm). These findings are in perfect agreement with an angular structure [14]. Because methylation of **7** with diazomethane provided a mixture of di-*O*-methyl- and tri-*O*-methylderivatives, the reliability of the negative Gibbs test was diminished. On the other hand, structure **7** was corroborated by the fact that exactly the same reaction mixture (mass spectrum and ^1H NMR evidence) re-

sulted from methylation of isojacareubin (**5**). Compound **7** is thus 1,6-dihydroxy-5-methoxy-6′,6′-dimethylpyrano-(2′,3′:3,4)-xanthone (5-*O*-methylisojacareubin), a new natural product.

The UV spectra of compounds **8**, **9** and **10** were almost identical and showed characteristics of a non-conjugated 1,3,5,6-tetrahydroxylated xanthone nucleus [13] exhibiting maxima at 250, 285 and 327 nm. While addition of $AlCl_3$ caused bathochromic shifts of about 70 nm, these shifts were reduced to +25 nm upon addition of HCl, indicating the existence of both chelated hydroxyl and ortho-dihydroxyl substituents in each compound. Sodium acetate induced bathochromic shifts of 40 nm (for **8**, **9** and **10**), which was indicative for hydroxyls at C-3 or/and C-6. Finally, addition of $NaOAc/H_3BO_3$ (+23 nm) confirmed the presence of ortho-dihydroxyl pairs in compounds **8**, **9** and **10**. Compounds **8–10** were optically active.

The EI-mass spectrum of compound **8** showed a low intensity $[M]^+$ at *m/z* 396, together with a base peak at *m/z* 273 $[M - C_9H_{15}]^+$, suggesting the presence of a monoterpenic substituent. The molecular formula was deduced as $C_{23}H_{24}O_6$. The ^1H NMR spectrum of compound **8** showed three hydroxyl groups (δ 13.19, HO-1; δ 9.50–8.50, 2 × OH). Two ortho-coupled protons were detected at δ 7.64 and 7.00 (*2d*, J = 8.8 Hz, H-7, H-8), together with a singlet at δ 6.31 (H-2) which was indicative of a 1,3,5,6-tetrahydroxylated xanthone. The presence of four multiplets at δ 5.10 (1H), 3.00 (2H), 2.72 (1H), and 2.20 (2H), respectively, a broad singlet at δ 4.57 (exo-CH_2) and three methyl singlets (δ 1.77, 1.61, 1.55) suggested the terpene to be a lavandulyl group [19]. Further evidence for the presence of a lavandulyl moiety was provided by the ^{13}C NMR data (Table 1), in particular an olefinic CH_2 at δ 111.5 and a CH at δ 47.8. The ^{13}C NMR signals were in complete accordance with data recently published for lavandulyl-substituted flavanones [19]. Gibbs reaction for compound **8a** was negative, indicating that the lavandulyl moiety is located at C-4. This was further confirmed by an important paramagnetic shift of H-2 in pyridine-d_5 (+0.44 ppm) [14]. The structure of compound **8** is identical to that proposed for calycinoxanthon D (1,3,5,6-tetrahydroxy-4-lavandulylxanthone), a compound isolated from *H. calycinum* [20]. No spectroscopic data have yet been published for this compound.

The molecular formula $C_{28}H_{32}O_6$ was deduced for compound **9** from EI-mass spectrometry ($[M]^+$ at *m/z* 464) and ^{13}C NMR spectral data. For compound **9**, the fact that the mass was 68 amu greater than compound **8** and the unchanged ^1H NMR spectrum of the xanthone nucleus, suggested the presence of a C_{15} alkyl moiety. Comparison of ^{13}C NMR data of compounds **8** and **9** revealed, in addition to great similarity of the spectra of both compounds (Table 1), replacement of the C-7′ methyl signal of compound **8** (δ 25.8) through a CH_2 (δ 40.4) and five additional peaks (δ 27.3, 125.2, 131.4, 17.7, and 25.8) characteristic of a 3,3-dimethylallyl unit. Comparison with published ^{13}C NMR data of geranyl [21] and lavandulyl [20] moieties confirmed the alkyl substituent of compound **9** to be a *trans*-sesquilavandulyl chain [22]. Additional evidence was given from the EI-mass spectrum exhibiting the loss of a single isoprene unit $[M - C_5H_8]^+$, a monoterpene moiety $[M - C_{10}H_{17}]^+$ and the splitting up of $C_{14}H_{23}$ $[M - C_{14}H_{23}]^+$ to give the prominent base peak at *m/z* 273. Attachment of the *trans*-sesquilavandulyl moiety to C-4 was inferred from the strong paramagnetic shift of H-2 in pyridine-d_5 (+0.45 ppm) [14] and a negative Gibbs test with the trimethyl ether derivative **9a** [15]. The structure of **9** was assigned as 1,3,5,6-tetrahydroxy-4-*trans*-sesquilavandulylxanthone. We propose the name roeperanone for this compound, which is, to our knowledge, the first xanthone reported to bear a C_{15} alkyl group.

Similar to calycinoxanthon (**8**), xanthone **10** showed a $[M]^+$ at *m/z* 396 and a base peak at *m/z* 273 $[M - C_9H_{15}]^+$. The molecular formula was deduced to be $C_{23}H_{24}O_6$, corresponding to a 1,3,5,6-tetrahydroxylated xanthone substituted with a monoterpene unit. The ^1H NMR spectrum of compound **10** showed a chelated hydroxyl (δ 13.92) and a singlet at δ 6.54, while for the B-ring two ortho-coupled signals at δ 7.60 and 6.97 (*2d*, J = 8.8 Hz, H-7, H-8) were observed. Among the remaining 17 protons, only two olefinic protons were detected, appearing as broad singlets at δ 4.77 and δ 4.67. In addition, two high-field methyls (δ 1.10 and δ 0.97) were observed. Therefore, common substituents such as geranyl or lavandulyl moieties could be excluded. The ^{13}C NMR spectrum confirmed the existence of a double bond and showed it to be terminal (δ 150.3 (*s*) and δ 108.3 (*t*)) (Table 1). Consequently, the terpene moiety had to be cyclized in order to comply with the molecular formula $C_{23}H_{24}O_6$. ^1H and ^{13}C NMR data of compound **10** were very similar to those reported for paxanthonin [23], recently isolated from a cell suspension culture of *H. patulum*. Slight differences were in agreement with the lack of a 5-*O*-methyl ether in compound **10**. Recording of the ^1H NMR spectrum of compound **10** in pyridine-d_5 did not significantly affect the shift of the proton at δ 6.54 (+0.05 ppm) suggesting it to be located at C-4 [14]. Further evidence for the attachment of the monoterpene unit at C-2 was given by a positive Gibbs reaction [15], carried out with the methylated derivative **10a**. Formation of a blue indophenol chromophore with λ_{max} = 707 nm [11] demonstrated the existence of an unsubstituted position *para* to HO-1. Hence, compound **10** was identified as 5-*O*-demethylpaxanthonin, a new natural compound.

Four of the constituents (**6**, **7**, **9**, **10**) of *H. roeperanum* isolated in the present study are new natural constituents. The prenylated xanthones **4–10** all possess a 1,3,5,6-oxygenation pattern, which is one of the predominating patterns within the Guttiferae. This agrees well with the observation of Bennet and Lee [3] who noted that the oxygenation pattern of alkylated xanthones is less diverse than that of simple oxygenated xanthones. Co-occurrence of simply oxygenated xan-

thones and more complex prenylated derivatives has been described in several species of the family Guttiferae [3, 20].

The isolated compounds were tested for their antifungal activity against *Candida albicans* and *Cladosporium cucumerinum* in TLC bioautographic assays [24, 25]. The minimum amount of xanthones **4**, **6**, **8**, **9** and **10** required to inhibit the growth of *C. albicans* on TLC plates was 1 μg, whereas xanthone **7** showed antifungal activity at 5 μg. All other xanthones were inactive at a level of 10 μg. The reference compounds amphotericin B and miconazole were active at 1 μg and 0.001 μg, respectively. It must be noted that the crude dichloromethane extract did not show activity against *C. albicans* at the usual test level of 100 μg, owing to the low concentration of the xanthones in *H. roeperanum*. None of the xanthones from *H. roeperanum* inhibited growth of *C. cucumerinum* at 10 μg. The isolated xanthones will be tested for IMAO activity.

EXPERIMENTAL

General. Mps: uncorr. UV: shift reagents prepared according to ref. [26]. ^{1}H and ^{13}C NMR: in acetone-d_6 (unless otherwise specified) at 200.06 MHz and 50.30 MHz, respectively; δ relative to TMS; multiplicities of ^{13}C signals obtained by DEPT experiments. EI-MS: 70 eV. Thermospray-MS (TSP-MS): NH_4OAc buffer; positive ion mode. TLC: precoated silica gel F_{254} aluminium sheets, with petrol–EtOAc–MeOH–H_2O (1:1:1:1, upper phase)–MEOH (9:1). Centrifugal partition chromatography (CPC): Pharmatech® CCC-1000 instrument, upper phase as mobile phase. Prep. HPLC: LiChrosorb RP-18 column (7 μm, 25 × 1.6 cm i.d.).

Plant material. Roots of *H. roeperanum* Schimp. ex A. Rich. were collected in Zimbabwe in January 1990 and identified by S. Mavi (National Herbarium, Harare, Zimbabwe). A voucher specimen is deposited at the National Herbarium of Zimbabwe.

Extraction and isolation. Ground, air-dried roots (400 g) were extracted at room temp. with CH_2Cl_2. Gel filtration of the CH_2Cl_2 extract (25 g) on Sephadex LH-20 with $CHCl_3$–MeOH (1:1) gave 3 frs (I–III). Further separation of fr. III (3.5 g) by gel filtration on Sephadex LH-20 with MeOH yielded a main fraction (3.050 g), which was dissolved in a mixture of hot (50°) upper phase (30 ml) and lower phase (30 ml) of petrol–EtOH–EtOAc–H_2O (9:7:3:2) in order to allow purification by CPC. Before injection, a solid (0.85 g) precipitated. After filtration and recrystallization from MeOH, the compound obtained was identified as betulinic acid by comparison with an authentic sample. The remaining filtrate (2.2 g) was sepd by CPC with petrol–EtOH–EtOAc–H_2O (9:7:3:2) to yield seven frs (A-G). After medium pres. LC (MPLC) on silica gel with petrol–EtOAc–MeOH–H_2O (1.5:1:1:1, upper phase), followed by prep. HPLC on RP-18 with MeOH–H_2O (88:12), and final purification by gel filtration on Sephadex LH-20 (MeOH), fr. D (207 mg) yielded compounds **6** (8.5 mg) and **7** (3 mg). Fr. E (520 mg) yielded compound **8** (18 mg) after MPLC on RP-18 with MeOH–H_2O (70:30), followed by gel filtration on Sephadex LH-20 (MeOH). Fr. F (363 mg) was separated by CPC with petrol–EtOAc–MeOH–H_2O (1:1:1:1) to give six frs (A′-F′). Fr. A′ (65 mg) provided compound **9** (6.8 mg) after prep. HPLC on RP-18 with MeOH–H_2O (87:13) and subsequent gel filtration on Sephadex LH-20 (MeOH). Fraction D′ (50 mg) yielded xanthone **2** (5.5 mg) by applying the same procedure, but with MeOH–H_2O (73:27) for prep. HPLC. Fraction E′ (46 mg) yielded compounds **1** (3.9 mg) and **3** (3.0 mg), after gel filtration on Sephadex LH-20 (MeOH). Fr. C′ (125 mg) was subjected to CPC with cyclohexane–EtOAc–MeOH–H_2O (1.5:1:1:1) to give 3 frs (A″-C″). Fr. A″ (28 mg) yielded compound **10** (7 mg), after gel filtration on Sephadex LH-20 (MeOH), whereas gel filtration on Sephadex LH-20 (MeOH) of fr. B″ (80 mg) provided a further 14 mg of **8**, 6 mg of **4** and 4 mg of **5**.

Methylation. Compounds (1 mg) were dissolved in a freshly prepared soln of CH_2N_2 in Et_2O. After 12 hr at room temp., products were recovered by evapn of the solvent.

2-*Hydroxyxanthone* (**1**). Yellow amorphous powder, mp 214–217°. TLC: R_f 0.48. UV and ^{1}H NMR identical to ref. [7]. ^{13}C NMR identical to ref. [8].

5-*Hydroxy*-2-*methoxyxanthone* (**2**). Pale yellow amorphous powder, mp 245–248°. TLC: R_f 0.44. UV identical to ref. [9]. ^{1}H NMR (DMSO-d_6): 10.33 (1H, *s*, HO-5), 7.65 (1H, *d*, J = 9.0 Hz, H-4), 7.62 (1H, *dd*, J = 7.7, 2.1 Hz, H-8), 7.57 (1H, *d*, J = 3.0 Hz, H-1), 7.48 (1H, *dd*, J = 9.0, 3.0 Hz, H-3), 7.32 (1H, *dd*, J = 7.7, 2.1 Hz, H-6), 7.24 (1H, *t*, J = 7.7 Hz, H-7), 3.89 (3H, *s*, MeO-2). ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 242 $[M]^+$ (100), 227 (26), 213 (11), 212 (22), 199 (6), 171 (22), 115 (12).

2,5-*Dimethoxyxanthone* (**2a**). Treatment of compound **2** with CH_2N_2 gave **2a**. ^{1}H NMR (DMSO-d_6): identical to ref. [10]. EI-MS *m/z* (rel. int.): 256 $[M]^+$ (100), 241 (35), 226 (15).

1,5-*Dihydroxy*-2-*methoxyxanthone* (**3**). Yellow amorphous solid, mp 160–164°. TLC: R_f 0.40. UV λ_{max}^{MeOH} nm (log ε) 206 (3.85), 257 (3.95), 320 (3.29), 374 (3.09); +NaOMe: 215, 255, 355, 425 (sh); +$AlCl_3$: 242, 274, 318, 352; +$AlCl_3$ + HCl: 240, 273, 315, 347; +NaOAc: 269, 355. ^{1}H NMR (DMSO-d_6): 12.70 (1H, *br s*, HO-1), 7.61 (1H, *dd*, J = 7.8, 1.9 Hz, H-8), 7.58 (1H, *d*, J = 9.2 Hz, H-3), 7.36 (1H, *dd*, J = 7.8, 1.9 Hz, H-6), 7.27 (1H, *t*, J = 7.8 Hz, H-7), 7.09 (1H, *d*, J = 9.2 Hz, H-4), 3.86 (3H, *s*, MeO-2). EI-MS *m/z* (rel. int.): 258 $[M]^+$ (100), 243 (47), 241 (9), 229 (15), 228 (8), 215 (59), 212 (15).

1,5,6 - *Trihydroxy* - 4′,5′ - *dihydro* - 4′,4′,5′ - *trimethylfurano*-(2′,3′:3,4)-*xanthone* (2-*deprenylrheediaxanthone B*) (**4**). Yellow gum, mp 125–130°. $[\alpha]_D^{20}$ 0 (Me_2CO; *c* 0.27). TLC: R_f 0.33. UV λ_{max}^{MeOH} nm (log ε) 251 (4.13), 327 (3.70); +NaOMe: 215, 251, 367; +$AlCl_3$: 269, 342, 396; +$AlCl_3$ + HCl: 260, 350;

+NaOAc: 225, 247, 364; +NaOAc + H_3BO_3: 227, 259, 328, 350. ^{1}H NMR (acetone-d_6): 13.43 (1H, *s*, HO-1), 7.58 (1H, *d*, J = 8.7 Hz, H-8), 6.95 (1H, *d*, J = 8.7 Hz, H-7), 6.10 (1H, *s*, H-2), 4.53 (1H, *q*, J = 6.6 Hz, H-2′), 1.59 (3H, *s*, H_3-4′), 1.36 (3H, *d*, J = 6.6 Hz, H_3-3′), 1.29 (3H, *s*, H_3-5′); $\Delta\delta = \delta$ (pyridine-d_5) − δ (acetone-d_6): H-8 (+0.42), H-7 (+0.27), H-2 (+0.42). ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 328 $[M]^+$ (35), 313 (100), 298 (12), 285 (15), 269 (6), 257 (7).

1-*Hydroxy*-5,6-*dimethoxy*-4′,5′-*dihydro*-4′,4′,5′-*trimethylfurano*-(2′,3′:3,4)-*xanthone* (5,6-O-*dimethyl*-2-*deprenylrheediaxanthone B*) (**4a**). Treatment of compounds **4** and **6** with CH_2N_2 gave **4a**. TSP-MS: 357 $[M + H]^+$. ^{1}H NMR (acetone-d_6): 13.31 (1H, *s*, HO-1), 7.96 (1H, *d*, J = 9.2 Hz, H-8), 7.25 (1H, *d*, J = 9.2 Hz, H-7), 6.18 (1H, *s*, H-2), 4.61 (1H, *q*, J = 6.6 Hz, H-2′), 1.64 (3H, *s*, H_3-4′), 1.43 (3H, *d*, J = 6.6 Hz, H_3-3′), 1.35 (3H, *s*, H_3-5′), 4.06 (3H, *s*, MeO-5), 3.98 (3H, *s*, MeO-6). Gibbs reaction [15]: negative.

1,5,6-*Trihydroxy*-6′,6′-*dimethylpyrano*-(2′,3′:3,4)-*xanthone* (*isojacareubin*) (**5**). Yellow gum, mp 170–175°. TLC: R_f 0.35. UV, ^{1}H NMR, ^{13}C NMR (Table 1) and EI-MS data identical with ref. [17]. Treatment of compound **5** with CH_2N_2 yielded a mixture of di-*O*-methyl- and tri-*O*-methylderivatives. TSP-MS *m/z* (rel. int.): 369 $[M + H]^+$ (80) and 355 $[M + H]^+$ (100).

1,6-*Dihydroxy*-5-*methoxy*-4′,5′-*dihydro*-4′,4′,5′-*trimethylfurano*-(2′,3′:3,4)-*xanthone* (5-O-*methyl*-2-*deprenylrheediaxanthone B*) (**6**). Pale yellow amorphous powder, mp 223–225°. $[\alpha]_D^{20}$ −15.4 (Me_2CO; *c* 0.6). TLC: R_f 0.49. UV λ_{max}^{MeOH} nm (log ε) 241 (3.81), 314 (3.89), 356 (3.81); +NaOMe: 214, 239, 263, 367; +$AlCl_3$: 232, 253, 268, 345, 400; +$AlCl_3$ + HCl: identical to $AlCl_3$ spectrum; +NaOAc: 223, 261, 374. ^{1}H NMR (acetone-d_6): 13.40 (1H, *s*, HO-1), 7.85 (1H, *d*, J = 8.6 Hz, H-8), 7.03 (1H, *d*, J = 8.6 Hz, H-7), 6.18 (1H, *s*, H-2), 4.60 (1H, *q*, J = 6.6 Hz, H-2′), 1.65 (3H, *s*, H_3-4′), 1.43 (3H, *d*, J = 6.6 Hz, H_3-3′), 1.35 (3H, *s*, H_3-5′), 4.02 (3H, *s*, MeO-5). $\Delta\delta = \delta$ (pyridine-d_5) − δ (acetone-d_6): H-8 (+0.28), H-7 (+0.2), H-2 (+0.36). ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 342 $[M]^+$ (37), 328 (15), 327 (100), 312 (15), 299 (7), 297 (6), 284 (6), 269 (10).

1,5-*Dihydroxy*-5-*methoxy*-6′,6′-*dimethylpyrano*-(2′,3′:3,4)-*xanthone* (5-O-*methylisojacareubin*) (**7**). Yellow gum, mp 201–205°. TLC: R_f 0.47. UV λ_{max}^{MeOH} nm (log ε) 251 (4.15), 319 (3.67), 367 (3.60); +NaOMe: 214, 255, 263, 339, 373; +$AlCl_3$: 214, 255, 276, 346, 416; +$AlCl_3$ + HCl: identical to $AlCl_3$ spectrum; +NaOAc: 209, 223, 261, 328, 374. ^{1}H NMR (acetone-d_6): 13.22 (1H, *br s*, HO-1), 7.84 (1H, *d*, J = 9.0 Hz, H-8), 7.05 (1H, *d*, J = 9.0 Hz, H-7), 6.90 (1H, *d*, J = 10.1 Hz, H-1′), 6.18 (1H, *s*, H-2), 5.81 (1H, *d*, J = 10.1 Hz, H-2′), 1.49 (6H, *s*, H_3-4′ and H_3-5′), 4.05 (3H, *s*, OMe-5); $\Delta\delta = \delta$ (pyridine-d_5) − δ (acetone-d_6): H-8 (+0.27), H-7 (+0.17), H-2 (+0.36), H-1′ (−0.12). ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 340 $[M]^+$ (36), 327 (26), 326 (15), 325 (100), 311 (8), 310 (56), 296 (12), 295 (83). Treatment of compound **7** with CH_2N_2 yielded a mixture of di-*O*-methyl- and tri-*O*-methylderivatives. TSP-MS *m/z* (rel. int.): 369 $[M + H]^+$ (40) and 355 $[M + H]^+$ (100).

1,3,5,6-*Tetrahydroxy*-4-*lavandulylxanthone* (*calycinoxanthon D*) (**8**). Yellow gum, mp 112–115°. $[\alpha]_D^{20}$ +4.9 (MeOH, *c* 0.57). TLC: R_f 0.37. UV, including shift reagents data identical to ref. [20]. ^{1}H NMR (acetone-d_6): 13.19 (1H, *s*, HO-1), 9.50–8.50 (2H, *br s*, 2 × OH), 7.64 (1H, *d*, J = 8.8 Hz, H-8), 7.00 (1H, *d*, J = 8.8 Hz, H-7), 6.31 (1H, *s*, H-2), 5.10 (1H, *m*, H-4′), 4.57 (2H, *br s*, H_2-9′), 3.00 (2H, *m*, H_2-1′), 2.72 (1H, *m*, H-2′), 2.20 (2H, *m*, H_2-3′), 1.77 (3H, *br s*, H_3-10′), 1.61, 1.55 (3H each *br s*, H_3-7′ and H_3-6′); $\Delta\delta = \delta$ (pyridine-d_5) − δ (acetone-d_6): H-8 (+0.38), H-7 (+0.24), H-2 (+0.44). ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 396 $[M]^+$ (3), 327 (4), 297 (2), 274 (12), 273 (100), 245 (9).

1-*Hydroxy*-3,5,6-*trimethoxy*-4-*lavandulylxanthone* (**8a**). Treatment of compound **8** with CH_2N_2 gave **8a**. TSP-MS: 439 $[M + H]^+$. ^{1}H NMR (acetone-d_6): 13.13 (1H, *s*, HO-1), 7.94 (1H, *d*, J = 9.1 Hz, H-8), 7.24 (1H, *d*, J = 9.1 Hz, H-7), 6.46 (1H, *s*, H-2), 5.07 (1H, *m*, H-4′), 4.60 (1H, *s*, H_a-9′), 4.54 (1H, *s*, H_b-9′), 2.96 (2H, *m*, H_2-1′), 2.74 (1H, *m*, H-2′), 2.16 (2H, *m*, H_2-3′), 1.76 (3H, *br s*, H_3-10′), 1.62, 1.55 (3H each *br s*, H_3-6′ and H_3-7′), 4.06 (3H, *s*, MeO-5), 4.01 and 3.98 (3H each, *s*, MeO-3 and MeO-6). Gibbs reaction [15]: negative.

1,3,5,6-*Tetrahydroxy*-4-trans-*sesquilavandulylxanthone* (*roeperanone*) (**9**). Yellow gum, mp 90–93°. $[\alpha]_D^{20}$ +6.2 (MeOH; *c* 0.54). TLC: R_f 0.41. UV λ_{max}^{MeOH} nm (log ε) 251 (4.38), 285 (3.86), 328 (4.04); +NaOMe: 241, 259; 383; +$AlCl_3$: 271, 394; +$AlCl_3$ + HCl: 257, 350; +NaOAc: 367; +NaOAc + H_3BO_3: 350. ^{1}H NMR (acetone-d_6): 13.20 (1H, *s*, HO-1), 9.50–8.50 (2H, *br s*, 2 OH), 7.63 (1H, *d*, J = 8.8 Hz, H-8), 7.00 (1H, *d*, J = 8.8 Hz, H-7), 6.30 (1H, *s*, H-2), 5.09 (2H, *m*, H-4′ and H-9′), 4.57 (2H, *br s*, H_2-9′), 3.00 (2H, *m*, H_2-1′), 2.73 (1H, *m*, H-2′), 2.21 (2H, *m*, H_2-3′), 1.95 (4H, *m*, H_2-7′ and H_2-8′), 1.78 (3H, *br s*, H_3-15′), 1.61 (3H, *br s*, H_3-11′), 1.55 (6H, *br s*, H_3-6′ and H_3-12′); $\Delta\delta = \delta$ (pyridine-d_5) − δ (acetone-d_6): H-8 (+0.39), H-7 (+0.24), H-2 (+0.45). ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 464 $[M]^+$ (5), 396 (2), 381 (3), 327 (5), 274 (113), 273 (100).

1-*Hydroxy*-3,5,6-*trimethoxy*-4-trans-*sesquilavandulylxanthone* (**9a**). Treatment of compound **9** with CH_2N_2 gave **9a**. TSP-MS: 507 $[M + H]^+$. ^{1}H NMR (acetone-d_6): 13.13 (1H, *s*, HO-1), 7.94 (1H, *d*, J = 9.1 Hz, H-8), 7.24 (1H, *d*, J = 9.1 Hz, H-7), 6.46 (1H, *s*, H-2), 5.10 (2H, *m*, H-4′ and H-9′), 4.61 (1H, *s*, H_a-9′), 4.56 (1H, *s*, H_b-9′), 2.96 (2H, *m*, H_2-1′), 2.68 (1H, *m*, H-2′), 2.20 (2H, *m*, H_2-3′), 1.96 (4H, *m*, H_2-7′ and H_2-8′), 1.76 (3H, *br s*, H_3-15′), 1.61 (3H, *br s*, H_3-11′), 1.55 (6H, *br s*, H_3-6′ and H_3-12′), 4.06 (3H, *s*, MeO-5), 4.01 and 3.99 (3H each, *s*, MeO-3 and MeO-6). Gibbs reaction [15]: negative.

1,3,5,6-*Tetrahydroxy*-2-(2′,2′-*dimethyl*-4′-*isopropenyl*)-*cyclopentanylxanthone* (5-O-*demethylpaxanthonin*) (**10**). Yellow powder, mp 142–146°

[MeOH]. $[\alpha]_D^{20}$ −28.6 (Me_2CO; *c* 0.36). TLC: R_f 0.29. UV λ_{max}^{MeOH} nm (log ε) 251 (4.32), 283 (3.83), 327 (4.02); +NaOMe: 242, 261, 382; +$AlCl_3$: 271, 390; +$AlCl_3$ + HCl: 254, 353; +NaOAc: 366; +NaOAc + H_3BO_3: 350. ^{1}H NMR (acetone-d_6): 13.92 (1H, *s*, HO-1), 7.60 (1H, *d*, J = 8.8 Hz, H-8), 6.97 (1H, *d*, J = 8.8 Hz, H-7), 6.54 (1H, *s*, H-4), 4.77 (1H, *br s*, H-7'b), 4.67 (1H, *br s*, H-7'a), 3.69 (1H, *dd*, $J_{1',5'b}$ = 7.6, $J_{1',5'a}$ = 11.0, H-1'), 3.08–2.92 (2H, *m*, H-4' and H-5'b), 1.78 (3H, *s*, H-8'), 1.74–1.68 (2H, *m*, H-3'a and H-5'a), 1.59 (1H, *t*, $J_{a,b} = J_{3'b,4'}$ = 12, H-3'b), 1.10 (3H, *s*, H_3-9'), 0.97 (3H, *s*, H_3-10'); $\Delta\delta = \delta$ (pyridine-d_5) − δ (acetone-d_6): H-8 (+0.45), H-7 (+0.30), H-4 (+0.05). ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 396 $[M]^+$ (23), 353 (16), 339 (9), 274 (15), 273 (100), 245 (9).

1-*Hydroxy*-3,5,6-*trimethoxy*-2-(2',2'-*dimethyl*-4'-*isopropenyl*)-*cyclopentanylxanthone* (**10a**). Treatment of compound **10** with CH_2N_2 gave **10a**. TSP-MS: 439 $[M+H]^+$. ^{1}H NMR (acetone-d_6) (incomplete data): 13.60 (1H, *s*, HO-1), 7.92 (1H, *d*, J = 9.1 Hz, H-8), 7.25 (1H, *d*, J = 9.1 Hz, H-7), 6.73 (1H, *s*, H-4), 4.77 (1H, *br s*, H-7'b), 4.68 (1H, *br s*, H-7'a), 1.78 (3H, *s*, H-8'), 1.07 (3H, *s*, H_3-9'), 0.91 (3H, *s*, H_3-10') 4.06 (3H, *s*, MeO-5), 4.02 and 3.98 (3H, each, *s*, MeO-3 and MeO-6). Gibbs reaction [15]: positive, λ_{max} 707 nm.

Acknowledgements—This work was supported by the Swiss National Science Foundation. Thanks are also extended to the Directorate for Development Co-operation and Humanitarian Aid (Swiss Federal Department of Foreign Affairs) and to the Fondation Herbette of the University of Lausanne.

REFERENCES

1. Exell, A. W. and Wild, H. (1961) *Flora Zambesiaca*, Vol. 1, 380. Crown Agents for Oversea Government and Administration, London.
2. Iwu, M. M. (1993) *Handbook of African Medical Plants*, p. 355. CRC Press, Boca Raton, Florida.
3. Bennet, G. J. and Lee, H. H. (1989) *Phytochemistry* **28**, 967.
4. Hostettmann, K. and Hostettmann, M. (1989) in *Methods in Plant Biochemistry*, Vol. 1 (Dey, P. M. and Harborne, J. B., eds), p. 493. Academic Press, London.
5. Rocha, L., Marston, A., Kaplan, M. A. C., Stöckli-Evans, H., Thull, U., Testa, B. and Hostettmann, K. (1994) *Phytochemistry* **36**, 1381.
6. Suzuki, O., Katsumata, Y., Oya, M., Chari, V. M., Klapfenberger, R., Wagner, H. and Hostettmann, K. (1978) *Biochem. Pharmacol.* **27**, 2075.
7. Cardona, L. and Seoane, E. (1982) *J. Nat. Prod.* **45**, 134.
8. Frahm, A. W. and Chaudhuri, R. K. (1979) *Tetrahedron* **35**, 2035.
9. Cardona, L., Fernández, I. and Pedro, J. R. (1992) *Heterocycles* **34**, 479.
10. Cardona, M. L., Pedro, J. R., Seoane, E. and Vidal, R. (1985) *J. Nat. Prod.* **48**, 467.
11. Lins Mesquita, A. A., De Barros Correa, D., Gottlieb, O. R. and Taveira Magalhaes, M. (1968) *Analyt. Chim. Acta* **42**, 311.
12. Venkataran, K. (1973) *Proc. Indian Natl Acad. Sci.* **39A**, 365.
13. Delle Monache, G., Botta, B., De Mello J. F., De Barros Coelho, J. S. and Menichini, F. (1984) *J. Nat. Prod.* **47**, 620.
14. Alves de Lima, R., Delle Monache, G. and Botta, B. (1982) *Rev. Latinoamericana Quim.* **13**, 61.
15. King, F. E., King, T. J. and Manning, L. C. (1957) *J. Chem. Soc.* 563.
16. Delle Monache, F., Botta, B., Nicoletti, M., de Barros Coêlho, J. S. and de Andrade Lyra, D. (1981) *J. Chem. Soc. Perkin Trans. 1*, 484.
17. Ishiguro, K., Nagata, S., Fukumoto, H., Yamaki, M., Isoi, K. and Oyama, Y. (1993) *Phytochemistry* **32**, 1583.
18. Miura, I., Hostettmann, K. and Nakanishi, K. (1978) *Nouv. J. Chim.* **2**, 653.
19. Inuma, M., Yokoyama, J., Ohyama, M., Tanaka, T. and Ruangrungsi, N. (1994) *Phytochemistry* **35**, 785.
20. Helboe, P. (1973) Thesis, *Xanthon Studier*, Kemisk Laboratorium B Danmarks farmaceutiske Højskole København 1973, Danmark, 92.
21. Ampofo, St. A. and Waterman, P. G. (1986) *Phytochemistry* **25**, 2351.
22. Schmaus, G., Schultze, W. and Kubecka, K.-H. (1989) *Planta Med.* **55**, 482.
23. Ishiguro, K., Nakajiama, M., Fukumoto, H. and Isoi, K. (1995) *Phytochemistry* **39**, 903.
24. Rahalison, L., Hamburger, M. and Hostettmann, K. (1991) *Phytochem. Anal.* **2**, 199.
25. Homans, A. L. and Fuchs, A. (1970) *J. Chromatogr.* **51**, 327.
26. Markham, K. R. (1982) *Techniques of Flavonoid Identification*. Academic Press, London.