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SOME CHEMICAL CONSTITUENTS ISOLATED FROM *ACORUS TATARINOWII* SCHOTT.

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NGUYEN THE DUNG[1], NGUYEN THI HONG VAN[1], PHAM DINH TY[1], W. C. TAYLOR[2]

[1]*Institute of Natural Products Chemistry, VAST, Vietnam*

[2]*School of Chemistry, University of Sydney, NSW 2006, Australia*

SUMMARY

A new 8,1′-neolignan, tatarinone, 4-[2-(1,2,3-trimethoxybenz-5-yl)-1-methylethyl]-2,5-dimethoxy-4-(2-propenyl)-2,5-cyclohexadien-1-one, has been isolated from Acorus tatarinowii rhizome and its structure determined by spectroscopic methods, including 2D-NMR spectra. Asarylaldehyde and a mixture of α-asarone and β-asarone were also isolated and indentified.

Keywords: *Acorus tatarinowii*; Araceae; 8,1′-neolignans; asarylaldehyde; α-asarone; β-asarone; NMR.

I - INTRODUCTION

Acorus tatarinowii Schott (Araceae) grows wild on the banks of mountain streams in Vietnam (Vietnamese name, Thach xuong bo), and is also found in China and India. The rhizomes are used as a herbal medicine with stomachic and sedative properties [1]. We have previously reported the isolation of 5-hydroxy-4-oxo-pentanoic acid from the rhizomes [2] and now report a new neolignan, tatarinone, **1**. Together with **1**, a two-isomer mixture of α-asarone **4** and β-asarone **5**, asarylaldehyde **6**, three known other compounds, were also reported.

II - EXPERIMENTAL

The melting point was determined on an Electrothermal AI9200 instrument; IR spectrum was recorded on an Impact-410-Nicolet instrument (Institute of Chemistry – VAST Vietnam); an EI-MS was recorded on a MS-Engine 5989B-HP instrument (Institute of Chemistry – VAST Vietnam) and EI-MS and HREI-MS were recorded on a Kratos MS25 RFA instrument (School of Chemistry – University of Brisbane, Australia); unless otherwise stated 1D- and 2D-NMR spectra were recorded for $CDCl_3$ solutions on a Bruker Avance 500 spectrometer (Institute of Chemistry – VAST Vietnam) and Bruker Avance 400 spectrometer (School of Chemistry – University of Sydney, Australia). The optical rotation on a $CHCl_3$ solution was measured with a Polar 2001 (Optical Activity Ltd.) instrument. GC-MS was recorded on instrument.

Acorus tatarinowii was collected from the Tam Dao National Park and identified by botanist Ha Quoc Hoan (Botanical Station - Tam Dao National Park) and botanist Nguyen Van Phu (Institute of Ecology and Biological Resources - VAST Vietnam). A voucher specimen was deposited at the Department of Botany, Institute of Ecology and Biological Resources, VAST Vietnam.

The powdered dry rhizome (0.5 kg) was extracted successively with *n*-hexane (3 x 1000 ml), ethyl acetate (3 x 1000 ml) and methanol (3 x 1000 ml) at room temperature. The ethyl acetate extract was evaporated in vacuum. On standing in a refrigerator for a week the

concentrate (26g) deposited crystals of 5-hydroxy-4-oxo-pentanoic acid [7]. The residue (25 g) was chromatographed on a silica gel column with *n*-hexane – ethyl acetate mixtures having increasing ethyl acetate content from 0% to 100%; the 100% ethyl acetate fractions, 91-100, contained **1**. Recrystallization from acetone gave **1** (100 mg). The 60% ethyl acetate fractions, 40-70, yielded **2**, **3** and **4**. **2** formed as tablet crystals after recrystallizing from acetone. The obtained mixture of **3** and **4** was a brown liquid.

Tartarinone, **1**, $C_{23}H_{30}O_6$, 4-[2-(1,2,3-trimethoxybenz-5-yl)-1-methylethyl]-2,5-dimethoxy-4-(2-propenyl)-2,5-cyclohexadien-1-one, colourless needles, mp. 178 - 179°C, $[\alpha]_D^{20}$ +17° (*c*, 0.5). IR (KBr): 3074, 2969, 2917, 1660, 1645, 1600, 1507, 1468, 1375, 1216, 1183, 1124, 1018, 859 cm-1; HREI-MS: m/z 402.2044, calc. for $C_{23}H_{30}O_6$, 402.2042. EI-MS 402 [$M^{\bullet+}$] (18%), 221, 209, 194, 181 (100%), 179, 166, 161, 148, 91, 77; ^{1}H-NMR and ^{13}C-NMR, HMQC, HMBC, COSY, NOESY: see table 1 and figure 1.

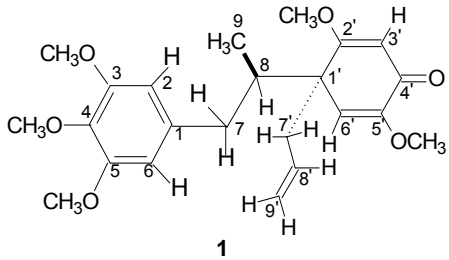
The mixture of α-asarone **4**, $C_{12}H_{16}O_3$, (E)-1-(1-allyl)-2,4,5-trimethoxybenzee, and β-asarone **5**, $C_{12}H_{16}O_3$, (Z)-1-(1-allyl)-2,4,5-trimethoxybenzene, a brown liquid mixture. IR (KBr): 2995, 2936, 2834, 1660, 1607, 1580, 1511, 1461, 1400, 1209, 1037, 866, 825, 757 cm-1; EI-MS 208 [$M^{\bullet+}$] (100%), 191, 181, 165, 131, 105, 91, 69; GC-MS: Rt (min.) 27.06 (β-asarone), 28.72 (α-asarone). The first set of signals (for α-asarone): ^{1}H-NMR ($CDCl_3$, TMS, 500 MHz), δH, ppm, (J, Hz): 1.88 q (6.8, 1.5), 3H (CH_3-9); 3.79 s, 3H (CH_3O at C-4); 3.84 s, 3H (CH_3O at C-2); 3.88 s, 3H (CH_3O at C-5); 6.09 m, 1H (H-8); 6.48 s, 1H (H-6); 6.65 q (15.8, 1.8) 1H (H-7); 6.94 s, 1H (H-3); ^{13}C-NMR ($CDCl_3$, TMS, 125MHz), δC, ppm: 18.48 (CH_3-9), 55.81 (CH_3O at C-5), 56.22 (CH_3O at C-4), 56.35 (CH_3O at C-2), 97.74 (C-6); 109.67 (C-3); 118.76 (C-1); 123.92 (C-8); 124.84 (C-7); 143.12 (C-5); 148.53 (C-2); 150.43 (C-4). The second set of signals (for β-asarone): ^{1}H-NMR ($CDCl_3$, TMS, 500 MHz), δH, ppm, (J, Hz): 1.84 q (7.2, 2.0), 3H (CH_3-9); 3.789 s, 3H (CH_3O at C-4); 3.83 s, 3H (CH_3O at C-2); 3.86 s, 3H (CH_3O at C-5); 5.75 m, 1H (H-8); 6.49 q (9.5, 1.8), 1H (H-7); 6.53 s, 1H (H-6); 6.84 s, 1H (H-3); ^{13}C-NMR ($CDCl_3$, TMS, 125 MHz), δC, ppm: 14.40 (CH_3-9), 55.81 (CH_3O at C-5), 56.14 (CH_3O at C-4), 56.39 (CH_3O at C-2), 97.41 (C-6), 114.00 (C-3), 117.85 (C-1), 124.59 (C-7), 125.40 (C-8), 142.18 (C-5), 148.37 (C-3), 151.33 (C-4); HMQC, HMBC, HH-COSY were recorded for the mixture.

Asaraldehyde **6**, $C_{10}H_{12}O_4$, 2,4,5-trimethoxybenzaldehyde, colourless tablet crystal, mp. 110 - 112; IR (KBr): 2931, 2860, 1660, 1608, 1519, 1479, 1408, 1357, 1291, 1267, 1217, 1126, 1023, 873, 751, 572 cm^{-1}; EI-MS: 196 [$M^{\bullet+}$] (100%), 181, 150, 125, 110, 95, 79, 69. ^{1}H-NMR ($CDCl_3$, TMS, 500 MHz), δH, ppm: 3.88 s, 3H (2- CH_3O); 3.93 s, 3H (5-CH_3O); 3.97 s, 3H (4-CH_3O); 6.50 s, 1H (H-3); 7.33 s, 1H (H-6); 10.32 s, 1H (-CH=O). ^{13}C-NMR ($CDCl_3$, TMS, 125 MHz), δC, ppm: 56.16 (2-CH_3O), 56.21 (5-CH_3O), 56.29 (4-CH_3O), 96.00 (C-3), 109.08 (C-6), 117.39 (C-1), 143.60 (C-2), 155.78 (C-5), 158.62 (C-4), 187.95 (-CH=O). HMQC, HMBC were recorded.

III - RESULTS AND DISCUSSION

Chromatography of an ethyl acetate extract of the dried rhizomes yielded tatarinone as colourless crystals, mp 178-9°. The molecular formula $C_{23}H_{30}O_6$ was established by HREIMS. The IR spectrum of **1** showed a strong absorption band at 1660 cm^{-1} from C=O and absorption bands at 1645, 1600 cm^{-1} from double bonds. The ^{1}H-NMR spectrum ($CDCl_3$) had distinct sets of signals consistent with the structure **1**. Signals for an allyl group occurred at δ 2.74 (H_a-7′) as a doublet of doublets of triplets ($J_{7'a,7'b}$ = 13 Hz, $J_{7'a,8'}$ = 7.8 Hz, $J_{7'a,9'a}$ = 1.2 Hz, $J_{7'a,9'b}$ = 1 Hz), δ 2.65 (H_b-7′) with a similar pattern ($J_{7'b,7'a}$ = 13 Hz, $J_{7'b,8'}$ = 6.5 Hz, $J_{7'b,9'a}$ = $J_{7'b,9'b}$ = 1 Hz), δ 5.48, m, (H-8′), 5.04 (H_a-9′), doublet of doublets of triplets ($J_{9'a,9'b}$ = 2 Hz, $J_{9'a,8'}$ = 17 Hz, $J_{9'a,7'a}$ = $J_{9'a,7'b}$ = 1.2 Hz), and δ 4.97 (H_b-9′), a doublet of doublets of triplets ($J_{9'b,9'a}$ = 2.0 Hz, $J_{9'b,8'}$ = 10.0 Hz, $J_{9'b,7'a}$ = $J_{9'b,7'b}$ = 1 Hz). Another coupling pattern arose from a CH_3CHCH_2- group: δ 3.05 (H_a-7), broad doublet of doublets ($J_{7a,7b}$ = 12.6 Hz, $J_{7a,8}$ = 2.1 Hz), δ

2.11 (H_b-7), doublet of doublets ($J_{7b,7a}$ = 12.6 Hz, $J_{7b,8}$ = 11.7 Hz), δ 2.20 (H-8) doublet of doublets of quartets ($J_{8,7a}$ = 2.1 Hz, $J_{8,7b}$ = 11.7 Hz, $J_{8,9}$ = 6.5 Hz), and δ 0.67 [$(H\text{-}9)_3$] (doublet, $J_{9,8}$ = 6.5 Hz). The COSY spectrum, in addition to supporting the major coupling relationships in **1**, showed cross peaks between H_a-7 and H_b-7 (weaker) and aromatic protons (2 H singlet) at δ 6.36 (H-2, H-6), which in turn had cross peaks with a six proton singlets at δ 3.87 (3-OMe, 5-OMe). Remaining signals in the ^{1}H-NMR spectrum of **1** were two olefinic singlets at δ 5.70 (H-3′) and δ 5.39 (H-6′), having cross peaks in the COSY spectrum with a singlet signal (6H) at δ 3.78 (2′-OMe, 5′-OMe, respectively).



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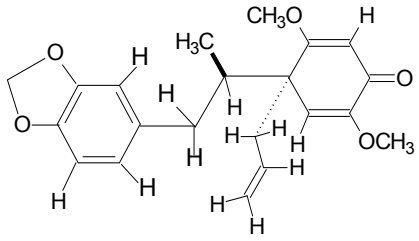

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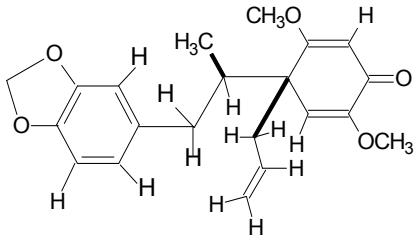

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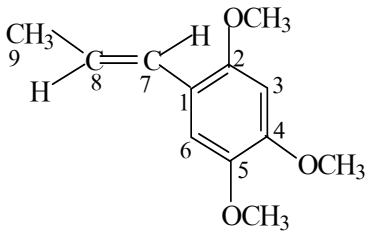

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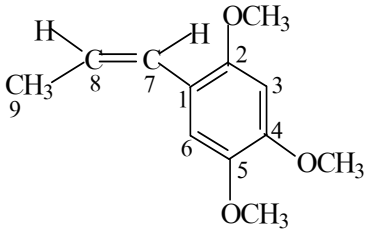

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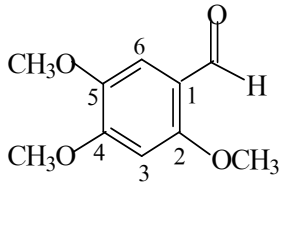

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The ^{13}C-NMR spectrum was assigned (table 1) on the basis of DEPT, HMQC, and HMBC spectra. Signals of protonated carbons were consistent with the groupings indicated by the ^{1}H-NMR data. The HMBC spectrum was especially valuable for assigning quaternary carbons. In particular, C-1′ had many correlations, with H_aH_b-7, H-8, $(H\text{-}9)_3$, H-3′, H-6′, H_aH_b-7′ and H-8′. These and other correlations (table 1) fully supported the structure **1** for tatarinone. The NMR data was similar to those reported for the diasteriomeric 8,1′ neolignans, hookerinone A (**2**) and B (**3**), isolated from *Piper hookeri* [3, 4], apart from differences due to the different substitution of the aromatic ring and rather large discrepancies in the ^{13}C shifts of C-2′ and C-4′ (166.3 and 200.2 respectively for **2** and 177.8 and 182.6 for **1**). In contrast the ^{13}C shifts of the dienone ring of **1** are in good accord with those reported for burchellin and related compounds [5].

The NOESY spectrum (Fig. 1) gave further support. Also, in $CDCl_3/C_6D_6$ (4:1), the ^{1}H NMR signals of 2′-OMe and 5′-OMe separated (δ 3.62 and 3.67 respectively) and selective 1-D gsNOE spectra could be obtained. Selective inversion of the H-3′ signal gave a selective NOE to 2′-OMe only. Inversion of the H-6′ signal gave a NOE to 5′-OMe, and also to H_aH_b-7, H-8, $(H\text{-}9)_3$ and H_aH_b-7′ in keeping with structure **1.** Also, inversion of $(H\text{-}9)_3$ gave NOE's to H-2/H-6, H_aH_b-7, H-8, 2′-OMe, H-3′, H-6′, and H_aH_b-7′.

Table 1: Assignment of ^{1}H-NMR and ^{13}C-NMR chemical shifts of tatarinone, **1**

C	^{13}C-NMR (100 MHz, $CDCl_3$, TMS)		^{1}H-NMR (400 MHz, $CDCl_3$, TMS)	HMQC	HMBC
	δC, ppm	DEPT	δH, ppm (*J*, Hz)		
1	2	3	4	5	6
1	136.2*	C	-	-	H-2, H-6, H_a-7, H_b-7, H-8
2	105.9	CH	6.36, s, H-2	H-2	H-6, 3-OCH_3, H_a-7, H_b-7
3	152.9	C	-	-	H-2, 3-OCH_3
4	136.2*	C	-	-	H-2, H-6, 4-OCH_3, H_a-7, H_b-7, H-8
5	152.9	C	-	-	H-6, 5-OCH_3
6	105.9	CH	6.36, s, H-6	H-6	H-2, 5-OCH_3 H_a-7, H_b-7
7	38.1	CH_2	3.05, dd ($J_{7a,7b}$ = 12.6, $J_{7a,8}$ = 2.0), H_a-7; 2.11, dd ($J_{7b,7a}$ = 12.6, $J_{7b,8}$ = 11.7), H_b-7	H_a-7, H_b-7	H-2, H-6, H-8, $(H\text{-}9)_3$
8	43.1	CH	2.20, m, H-8	H-8	H_a-7, H_b-7, H_a-7', H_b-7', $(H\text{-}9)_3$
9	14.4	CH_3	0.67, d ($J_{9,8}$ = 6.5) $(H\text{-}9)_3$	$(H\text{-}9)_3$	H_a-7, H_b-7, H-8
1'	51.0	C	-	-	H-3', H-6', H-8', H_a-7, H_b-7, H_a-7', H_b-7', H-8, $(H\text{-}9)_3$
2'	177.8	C	-	-	H-3', H-6', 2'-OCH_3, H_a-7', H_b-7', H-8
3'	104.2	CH	5.70, s, H-3'	H-3'	H-3', H-6', H-8, 2'-OCH_3
4'	182.6	C	-	-	H-3', H-6'

1	2	3	4	5	6
5’	152.2	C	-	-	H-3’,H-6’, H_a7’, 5’-OCH_3
6’	112.3	CH	5.39, s, H-6’	H-6’	H-3’,H_a-7’,H_b-7’, H-8, H-8’, 5’-OCH_3
7’	41.5	CH_2	2.74, ddt ($J_{7'a,7'b}$ = 13.0, $J_{7'a,8'}$ = 7.8, $J_{7'a,9'a}$ = 1.2, $J_{7'a,9'b}$ = 1), H_a-7’; 2.65, ddt ($J_{7'b,7'a}$ = 13.0, $J_{7'b,8'}$ = 6.5, $J_{7'a,9'a}$ = $J_{7'a,9'b}$ = 1), H_b-7’	H_a-7’, H_b-7’	H_a-7’, H_b-7’, H-8’, H-6’, H_a-9’, H_b-9’
8’	132.6	CH	5.48, m, H-8’	H-8’	H_a-7’, H_b-7’, H-3’, H-6’, H_a-9’, H_b-9’
9’	117.8	$=CH_2$	5.04, ddt ($J_{9'a,8'}$ = 17.0, $J_{9'a,9'b}$ =2.0, $J_{9'a,7'a}$ = $J_{9'a,7'b}$ = 1), H_a-9’; 4.97, ddt ($J_{9'b,8'}$ = 10.0, $J_{9'b,9'a}$ = 2.0, $J_{9'b,7'a}$ = $J_{9'b,7'b}$ = 1), H_b-9’	H_a-9’, H_b-9’	H_a-7’, H_b-7’
4-OCH_3	60.7	CH_3	3.83, s, CH_3		
3-OCH_3 5-OCH_3	56.0	CH_3	3.87, s, 2xCH_3		
2’-OCH_3	55.7	CH_3	3.72, s, 2xCH_3		
5’-OCH_3	55.0	CH_3			

*^{13}C-NMR signals of the C-1 and C-4 in the 125 MHz spectra were separate at 136.32 and 136.20 ppm, respectively.

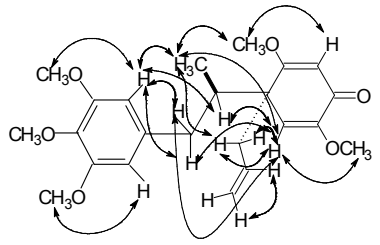

Fig. 1: NOESY correlations of **1**

In the EIMS a major fragment ion is at *m/z* 181, corresponding to the trimethoxybenzyl cation, ‘**a**’; the alternative ion from this fragmentation is ion, ‘**b**’ (*m/z* 221). Cleavage of the 8-1 bond gives the ion ‘**c**’ (*m/z* 209); if this occurs with H-transfer the ion ‘**d**’ (*m/z* 194) can form (Fig. 2).

Accepting the arguments used by Pradhan et al. [4] to assign the relative stereochemistry of the two diasteromers, hookerinone A (**2**) and B (**3**), as (8*R**,1′*R**) and (8*R**,1′*S**) respectively, the stereochemistry of tatarinone is (8*R**,1′*R**) as shown in **1**. This is suggested by the chemical shifts of $(H\text{-}9)_3$, δ_H 0.66 and δ_C14.4, which are more in agreement with those of hookerinone A. However, a more satisfactory comparison would be possible only if both

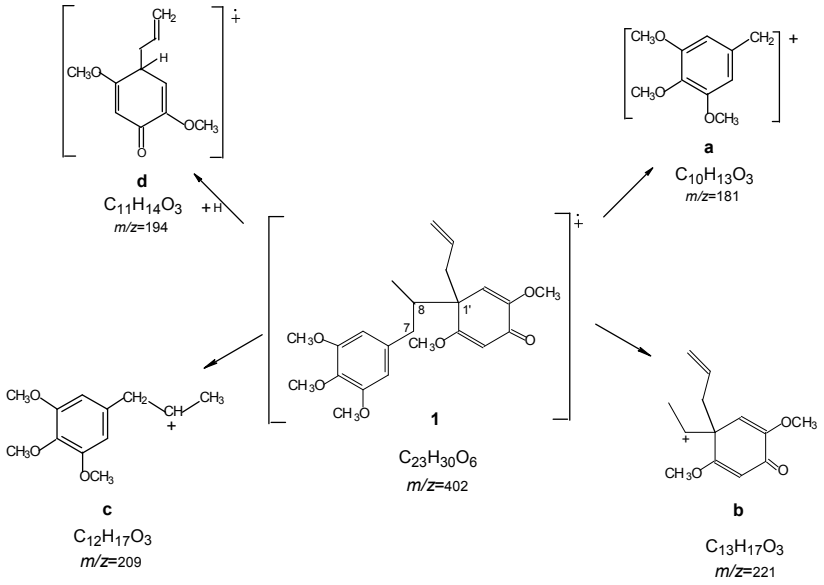

Figure 2: Mass spectral fragmentation of tatarinone **1**

isomers of tatarinone were available. The use of NOE results for assigning stereochemistry in this situation is difficult because of conformational mobility around the C-8-C-1′ bond.

The neolignan, tatarinone, is presumably the product of phenolic coupling in an 8-1′ fashion of two arylpropanoid precursors having 3,4,5- and 2,4,5-oxygenation patterns (e.g. isoelemicin and γ-asarone types, respectively). α-Asarone and β-asarone are major components of *Acorus calamus* and other *Acorus* species, including *A. tatarinowii* [6, 7], but γ-asarone is also a constituent [8]; elemicin and isoelemicin have also been found in various *Acorus* spp. [6, 9]. 8,1′- Neolignans have commonly been isolated from Lauraceae [10] and Piperaceae [11] spp., but typically products of the initial coupling have undergone further reactions to produce more complex structures such as the hydrobenzofuran type as found in burchellin etc. This is the first time that a 8,1′ neolignan has been isolated from *Acorus*.

Besides tatarinone, from the above ethyl acetate extract of the dried rhizomes the chromatography yielded asarylaldehyde and a mixture of α-asarone and β-asararone. Their spectral data corresponded to the previously published data [12, 13]. The ^{1}H-NMR and ^{13}C-NMR spectra of the above mixture revealed two sets of signals corresponding to α-asarone and β-asararone. Based on [14], it was shown that GC-MS data of the mixture have supported identification of its constituents.

REFERENCES

1. Vo Van Chi. Dictionary of Medicinal Plants of Viet Nam (in Vietnamese), 1121 (1997).
2. Nguyen Thi Hong Van, Pham Dinh Ty, Nguyen The Dung, Ngo Thi Thuan, W. C. Taylor. Vietnam J. Chem., 40, P. 65 - 67 (2000).
3. P. Pradhan, S. J. Desai, L. P. Badheka, A. Banerji. Natural Product Letters, 4, 35 - 42 (1994).
4. P. Pradhan, A. Banerji. Phytochemical Analysis, 9, 71 - 74 (1998).
5. E. Wenkert, H. E. Gottlieb, O. R. Gottlieb, M. O. da S. Pereira, M. D. Formiga. Phytochemistry, 15, 1547 - 1551 (1976).
6. Y. Huang, Z. He, Y. Cao, J. Wu. Sepu, 267 - 270 (1993).

7. R. Xiang, C. Fan. Zhongcaoyao, 14, 41 - 44 (1983).
8. R. Xiang, C. Fan. Zhongyao Tongbao, 8, 31-2 (1983).
9. H. Q. Wu, G. -Y. Zhang, Z. –Y. Zhang, Z. –J. Lei. Fenxi Ceshi Xuebao, 19, 70 - 71 (2000).
10. O. R. Gottlieb. Progress in the Chemistry of Natural Products (W. Herz, H. Grisebach, and G. Kirby Eds.), Vol. 35, 1 - 72. New York: Springer (1978).
11. V. S. Parmar, S. C. Jain, K. S. Bisht, R. Jain, P. Taneja, A. Jha, O. Tyagi, A. K. Prasad, J. Wengel, C. E. Olsen, P. M. Boll. Phytochemistry, 46, 597 - 673 (1997).
12. Patra, A. et al. J. Nat. Prod., 44, 668 (1981).
13. Jacobson, M. et al. J. Nat. Prod., 39, 4121 (1976).
14. R. Oprean, M. Tamas, L. Roman. J. Pharm. Biomed. Anal., 18, 227 - 234 (1998).