Tetrahedron Vol. 49, No. 16, pp. 3425-3432, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Cytotoxic 9,10-Dihydrophenanthrenes from *Juncus effusus* L.

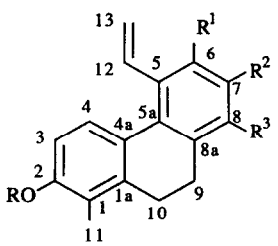
Marina Della Greca, Antonio Fiorentino, Lorenzo Mangoni, Antonio Molinaro, Pietro Monaco and Lucio Previtera*

Dipartimento di Chimica Organica e Biologica, Università Federico II, Via Mezzocannone 16, I-80134 Napoli, Italy

(*Received in UK* 16 *December* 1992)

Abstract. Nine 9,10-dihydrophenanthrenes, seven of them described for the first time, have been isolated in a further investigation of *Juncus effusus*. The structures have been defined on the basis of the spectral properties of the compounds. All the dehydrophenanthrene metabolites present in the plant have been tested for their cytotoxic properties and many of them have been found to have a good *in vitro* activity.

Phenanthrenes represent a rather uncommon class of aromatic metabolites which are presumably formed by oxidative coupling of the aromatic rings of stilbene precursors. In a recent phytochemical investigation of the aquatic plant *Juncus effusus* L. we have described nine 9,10-dihydrophenanthrenes **1** - **9**, characterized by a methyl at C-1, an oxygenated function at C-2 and a vinylic group at C-5[1]. In effect juncusol (**1**) and effusol (**2**) have already been isolated from the same source[2] and juncunol (**3**) has been reported as the main component of *Juncus roemeranius* [3].

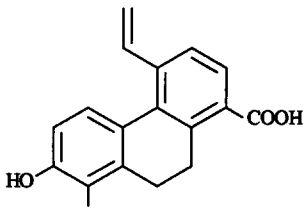


1 R=H R[1]=Me R[2]=OH R[3]=H
2 R=H R[1]=H R[2]=OH R[3]=H
3 R=H R[1]=H R[2]=Me R[3]=H
4 R=H R[1]=OH R[2]=Me R[3]=H
5 R=H R[1]=H R[2]=OH R[3]=Me
6 R=H R[1]=Me R[2]=H R[3]=OH
7 R=Me R[1]=Me R[2]=H R[3]=OH
8 R=H R[1]=H R[2]=OMe R[3]=Me
9 R=H R[1]=H R[2]=COOH R[3]=H

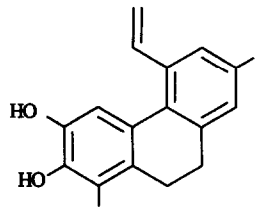
In the light of the potential antitoumoral activity of some of these compounds, evidenced by means of potato discs infected with *Agrobacterium tumefaciens* [4], *Juncus effusus* was completely examined and in this paper further nine dehydrophenanthrenes **10** - **18** are described together with the results of a screening of the biological activity and the isolation procedures of all the dehydrophenanthrenes from the plant.

Compound **10** was attributed the structure 8-carboxy-2-hydroxy-1-methyl-5-vinyl-9,10-dihydrophenanthrene. Its HR-EIMS spectrum gave a molecular peak at *m/z* 280.1119, according to the molecular formula $C_{18}H_{16}O_3$, and its IR spectrum showed the presence of a hydroxyl and a carboxyl function with absorptions at 3350, 3200 and 1685 cm^{-1}. In the ^1H-NMR spectrum were evidenced two aromatic AB systems

at δ 6.85, 7.55 and at δ 7.67, 7.78, a vinylic system at δ 5.13, 5.48 and 7.19, a methyl singlet at δ 2.20 beside a multiplet centred at δ 2.83, attributed to the H-9 and H-10 protons, which in a nOe experiment gave interaction with the methyl. In the H-C one bond COSY the proton at δ 6.85 was related to the carbon at δ 114.0 in agreement with its *ortho* position to a hydroxyl group and accordingly, the proton at δ 7.55, coupled to the above proton, gave a correlation with the hydroxyl bearing carbon at δ 156.6 in the H-C long range COSY.



10



11

This latter was also correlated to the methyl protons allowing the assignement of the protons at δ 6.85 and 7.55 to the C-3 and C-4 positions respectively and that of the methyl and the hydroxyl groups at the C-1 and C-2 positions.

Table 1. ^{1}H-NMR Data of Compounds **10** - **18**.

H	**10**[a]	**11**[b]	**12**[b]	**13**[b]	**14**[a]	**15**[a]	**16**[b]	**17**[a]	**18**[a]
3	6.85 d (8.4)		6.68 d (8.2)	6.77 d (8.4)	6.76 d (8.3)	6.81 d (8.4)	6.71 d (8.4)	6.77 d (8.6)	6.75 d (9.0)
4	7.55 d (8.4)	7.46 s	6.84 d (8.2)	6.86 d (8.4)	6.94 d (8.3)	7.21 d (8.4)	7.01 d (8.4)	7.07 d (8.6)	7.60 d (9.0)
5									7.10 s
6	7.78 d (8.2)	7.23 s		7.36 s				6.90 s	
7	7.67 d (8.2)								
8		7.05 s	7.17 s		7.30 s	7.00 s	7.00 s		6.88 s
9									
10	2.83 m	2.80 m	2.75 m	2.80 m	2.73 m	2.84 s	2.72 s	2.83 s	2.68 s
11	2.20s	2.35 s	2.28 s	2.30 s	2.29 s	2.21 s	2.27 s	2.21 s	2.16 s
12	7.19 dd (11.6 17.8)	6.99 dd (10.8 17.3)		9.70 s	9.95 s	5.73 q (6.3)	5.14 q (6.2)	5.63 q (6.3)	
	5.48 dd (2.0 11.6)	5.22 dd (1.4 10.8)							
13			2.08 s			1.43 d (6.3)	1.76 d (6.2)	1.65 d (6.3)	
	5.13 dd (2.0 17.8)	5.65 dd (1.4 17.3)							
Me		2.20 s	2.38 s	2.32 s	2.30 s	2.21 s	2.27 s	2.16 s	2.16 s
OMe				3.93 s			3.21 s		

[a] Recorded in acetone-d_6. [b] Recorded in $CDCl_3$

Moreover in the long range heterocorrelated spectrum the H-4 proton, the doublet proton at δ 7.78 and the vinylic proton at δ 7.19 were correlated to the same carbon at δ 135.2, which was assigned to C-5a. These correlations located univocally the vinylic chain at C-5, the proton doublet at C-6 and consequently the carboxyl group at C-8.

Compound **11** was attributed the structure 2,3-dihydroxy-1,7-dimethyl-5-vinyl-9,10-dihydrophenanthrene. The HR-EIMS spectrum showed the molecular ion at *m/z* 266.1324 according to the molecular formula $C_{18}H_{18}O_2$ and in the ^{1}H-NMR spectrum (Table 1) were present three aromatic singlet protons at δ 7.46, 7.23 and 7.05, two aromatic methyls at δ 2.35 and 2.20, the vinylic protons at δ 6.99, 5.65 and 5.22 and the aliphatic methylenes at δ 2.80.

The proton singlet at δ 7.46 was attributed to the C-4 position on the basis of the chemical shift of the related carbon at δ 118.9 and of its long range correlation to the hydroxyl bearing carbon at δ 150.5, which was also related to the methyl protons at δ 2.35. This latter group was then located at C-1 on the basis of the nOe interaction with the aliphatic methylene at δ 2.80. The heterocorrelation of all the aromatic protons as well as that of the vinylic proton at δ 6.99 with the same carbon at δ 130.7 and the nOe interaction of both the aromatic protons at δ 7.23 and 7.05 with the methyl at δ 2.20 univocally assigned the vinyl chain at C-5 and the methyl group at C-7.

Compound **12** had a molecular formula $C_{18}H_{18}O_3$ according to the molecular ion at *m/z* 282 in its EIMS spectrum and showed in the IR spectrum absorptions at 3330 and 1685 cm^{-1} of hydroxyl and carbonyl groups. In the ^{1}H-NMR spectrum were present a singlet at δ 7.17, an AB system at δ 6.84 and 6.68, three methyls at δ 2.38, 2.28 and 2.08 and the H-9 and H-10 methylenes at δ 2.75. These data and the ^{13}C-NMR chemical shifts allowed to identify **12** as juncunone (5-acetyl-2,6-dihydroxy-1,7-dimethyl-9,10-dihydrophenanthrene), already isolated from *Juncus roemerianus* [5].

Table 2. ^{13}C-NMR Chemical Shifts (δ) of Compounds **10** - **18**.

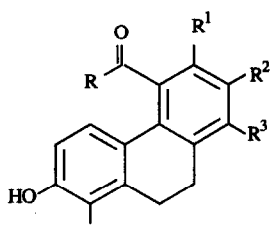
C	**10**[a]	**11**[b]	**12**[b]	**13**[b]	**14**[a]	**15**[a]	**16**[b]	**17**[a]	**18**[a]
1	121.9	121.9	121.3	121.3	122.7	122.1	120.9	122.1	122.1
2	156.6	150.5	155.9	156.4	156.9	155.3	153.6	155.4	155.2
3	114.0	140.4	112.6	112.4	113.1	112.7	111.6	112.3	113.6
4	124.0	118.9	128.5	129.4	130.3	126.3	128.8	127.2	130.6
5	138.8	135.2	120.2	133.4	116.9	125.2	122.1	130.6	110.0
6	122.7	127.7	154.2	106.2	160.5	148.9	152.8	128.9	155.1
7	128.9	136.0	124.4	154.1	123.9	126.1	126.9	127.0	122.4
8	129.4	127.5	133.9	129.9	137.4	129.3	126.4	155.6	122.3
9	26.9	29.9	31.8	25.2	28.7	30.6	29.9	30.7	29.9
10	25.7	25.6	28.2	25.5	26.2	26.8	26.4	27.0	26.2
11	11.5	12.2	11.6	11.5	11.8	11.9	11.8	11.9	11.6
12	136.6	138.7	207.9	193.2	197.7	69.1	58.9	68.6	
13	119.1	114.5	25.5			25.0	20.9	22.9	
1a	140.0	129.1	139.0	139.4	140.9	141.2	141.1	141.8	137.9
4a	126.7	127.2	127.8	124.7	124.0	124.9	124.5	124.3	127.3
5a	135.2	130.7	135.0	131.3	139.0	133.6	133.3	132.8	134.5
8a	139.2	138.5	129.5	139.3	130.1	131.4	130.3	126.2	127.4
Me		21.1	15.8	12.3	15.1	15.6	15.7	15.8	15.8
OMe			55.7			56.7			
CO	169.3								

[a]Recorded in acetone-d_6 [b]Recorded in $CDCl_3$

Compounds **13** and **14** were characterized by a formyl group at C-5 and were attributed the structure 5-formyl-2-hydroxy-1,8-dimethyl-7-methoxy-9,10-dihydrophenanthrene and 5-formyl-2,6-dihydroxy-1,7-dimethyl-9,10-dihydrophenanthrene respectively.

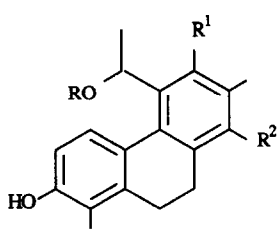
Compound **13** had a molecular formula $C_{18}H_{18}O_3$ according to the molecular ion at *m/z* 282.1266 in its HR-EIMS spectrum. The formyl group was evidenced by the proton singlet at δ 9.70 in the ^{1}H-NMR spectrum

and by the carbonyl carbon at δ 193.2 in the ^{13}C-NMR spectrum, beside the absorption at 1705 cm^{-1} in the IR spectrum. In the ^{1}H-NMR were also present two coupled doublets at δ 6.77 and 6.86 which were assigned to the H-3 and H-4 protons. The chemical shift at δ 112.4 of the carbon bearing the proton at δ 6.77 and the long range heterocorrelation of both the methyl protons at δ 2.30 and the doublet at δ 6.86 with the carbon bearing the hydroxyl group at δ 156.4 justified such an attribution. The proton at δ 7.36 gave nOe with the methoxyl methyl at δ 3.93 and the methyls at δ 2.30 and 2.32 gave nOe with the aliphatic methylenes at δ 2.80. These interactions univocally assigned the methyl at C-8, the methoxyl at C-7 and consequently the formyl at C-5.

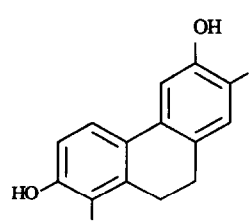


12 R = Me R^1 = OH R^2 = Me R^3 = H
13 R = H R^1 = H R^2 = OMe R^3 = Me
14 R = H R^1 = OH R^2 = Me R^3 = H

A molecular ion at *m/z* 268.1107 justified the molecular formula $C_{17}H_{16}O_3$ for compound **14.** The IR spectrum showed the carbonyl absorption at 1708 cm^{-1} and in the ^{1}H-NMR spectrum were present the formyl proton at δ 9.95, two AB doublets at δ 6.76 and 6.94, a singlet at δ 7.30, two methyls at δ 2.29 and 2.30 besides the aliphatic methylenes at δ 2.73. The nOe experiments evidenced interactions between the methyl at δ 2.29 and the aliphatic methylenes and between the methyl at δ 2.30 and the aromatic singlet at δ 7.30. Moreover in the dimethoxy derivative, obtained by Me_2SO_4 treatment, one of the methoxyl groups gave nOe with an aromatic doublet while the other gave nOe with the formyl proton.



15 R = R^2 = H R^1 = OH
16 R = Me R^1 = OH R^2 = H
17 R = R^1 = H R^2 = OH



18

Compound **15** had a molecular formula $C_{18}H_{20}O_3$ according to an ion at *m/z* 284.1401 in the HR-EIMS spectrum. In the ^{1}H-NMR spectrum besides two AB doublets at δ 6.81 and 7.21, a singlet at δ 7.00, two methyls as a singlet at δ 2.21 and the aliphatic methylenes at δ 2.84, were present a methyl doublet at δ 1.43 and a carbinol proton as a quartet at δ 5.73. These latter two signals evidenced the presence of a CH_3CHOH substituent in the molecule which was tentatively located at C-5 on the basis of biogenetic considerations. The H-C one bond and the H-C long range heterocorrelations allowed to assign the doublets at δ 6.81 and 7.21 at the C-3 and C-4 positions respectively and one of the methyls at δ 2.21 at the C-1 carbon. The nOe interaction of the methyl at δ 2.21 with the singlet at δ 7.00 and the H-C long range heterocorrelations of both this methyl and the carbinolic proton with the hydroxyl bearing carbon at δ 148.9 located the carbinolic chain at C-5 and the hydroxyl and methyl groups at C-6 and C-7 respectively. This pattern of substituents was identical to that of **12** and, accordingly, LAH reduction of this latter gave **15**.

Compound **16** was identified as the methyl ether derivative at C-12 of **15**. In the HR-EIMS spectrum was present a molecular ion at *m/z* 298.1575 according to $C_{19}H_{22}O_3$ and in the ^{1}H-NMR spectrum was present a methoxyl methyl at δ 3.21 beside the carbinol quartet at δ 5.14, the methyl doublet at δ 1.76, two methyl singlets at δ 2.27, two AB doublets at δ 6.71 and 7.01, a singlet at δ 7.00 and the aliphatic methylenes at δ 2.72. A comparison of these data as well as the ^{13}C-NMR chemical shifts with those of **15** showed an upfield shift of the H-12 proton as well as of the C-12 and C-13 carbons and a downfield shift of the H-13 proton, according to the structure.

Compound **17** was an isomer of **15** according to the EIMS and ^{13}C-NMR data. The ^{1}H-NMR spectrum showed the carbinol proton at δ 5.63 and the methyl doublet at δ 1.65 beside two AB doublets at δ 6.77 and 7.07, an aromatic singlet at δ 6.90, two methyls at δ 2.16 and 2.21 and the aliphatic methylenes at δ 2.83. The nOe interaction of the methyl at δ 2.21 with the methylene at δ 2.83 and the similarity of the C-1, C-2, C-3 , C-4, C-1a and C-4a chemical shifts in **17** and **15** justified the same pattern of substituents in the ring A. Moreover the chemical shift at δ 128.9 of the carbon linked to the proton at δ 6.90 excluded an *ortho* relation between the proton and the hydroxyl group while a nOe experiment evidenced the proximity of the proton to the methyl at δ 2.16 thus justifying the assignement of the methyl and the hydroxyl groups at C-7 and C-8 respectively. Compounds **15** - **17** were then found to be racemic mixtures on the basis of their CD and rotation data.

Compound **18** had a molecular ion at *m/z* 240 in the EIMS spectrum according to $C_{16}H_{16}O_2$ and showed in the ^{1}H-NMR spectrum two AB doublets at δ 6.75 and 7.60, two *meta* coupled protons at δ 6.88 and 7.10, two methyls at δ 2.16 and the four methylene protons at δ 2.68. These data were identical to those of the already known micrandol-B[6].

From a biogenetic point of view the metabolites **12** - **14** may be considered to derive from precursors with a vinylic chain at C-5. An oxidation of this chain might transform **4** in **12** as well as its oxidative cleavage might give **13** and **14** starting from **8** and **4** respectively. Also **15** - **17** may be located in the same pathway as a reduction of **12** might generate **15** and **16**.

The biological properties of all the 18 dehydrophenanthrenes were tested through the brine shrimp lethality assay[7] and the antitumor potato disc assay[5], although this latter was only conducted on the most abundant compounds. The results of the bioassays are reported in Table 3. The brine shrimp (*Artemia salina*) assays were performed in DMSO (1% final volume) using 10 x 3 animals/dose suspended in artificial sea water (5 ml) as previously reported[8]. The simple artificial sea water with 1% DMSO was used as a control. After 24 hr the data obtained were analyzed with the Finney program[9] which yielded LC_{50} values. All the compounds were

found to be toxic and the most active compounds were those with a hydroxyl function in the ring C and the vinylic chain or the carbinolic group at C-5.

Table 3. Biological Activity of Dehydrophenanthrenes **1** - **18**.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Brine Shrimp Assay LC_{50} µg/ml	4.6	5.6	25.3	3.0	3.0	4.0	3.0	11.2	14.1
Potato Disc Assay % inhibition	80	20	In	80	85	N.T.	N.T.	N.T.	N.T.
Compound	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Brine Shrimp LC_{50} µg/ml	15.7	16.5	84.9	81.5	83.0	8.0	16.1	5.0	6.5
Potato Disc Assay % inhibition	N.T.	N.T.	N.T.	N.T.	27	N.T.	N.T.	In	N.T.

N.T. not tested. In. inactive

The potato disc antitumor assay was performed using 2 mg of each compound dissolved in DMSO (0.5 ml), diluted in sterile water (1.5 ml) and added to a suspension of *Agrobacterium tumefaciens* (2 ml). An aliquot of the final suspension (25 µl) was added to sterile potato discs and the tumors were counted after 16 - 18 days. Also in this test the most active compounds were those with the vinylic chain at C-5.

EXPERIMENTAL

General procedures. NMR spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C on a Bruker AC 400 spectrometer. One bond and long range H-C COSY experiments were performed with the XHCORR microprogramme using delays corresponding to $J_{C,H}$ 160 Hz and 8 Hz respectively. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. EI mass spectra were obtained with a Kratos MS 80 apparatus. The CD spectra were recorded in EtOH solutions on a Jasco J-5OO apparatus. IR spectra were determined in $CHCl_3$ solutions on a FT-IR Perkin-Elmer mod. 1740 spectrometer.

Isolation of compounds. J effusus, collected in the Summer near Naples was air dried and extracted with ethyl ether to give crude material (125 g) which was separated by conventional procedures into an acidic (34 g) and a neutral fraction (85 g). The neutral fraction was chromatographed on silica gel and the fractions eluted with hexane Et_2O (9 : 1), after preparative tlc (hexane - benzene 2 : 3) gave pure juncunol (**3**) (66 mg). Elution with hexane - Et_2O (17 : 3) gave **11**, purified by preparative tlc (hexane -AcOEt 13 : 7) (4 mg): ν_{max} 3340 and 1603 cm^{-1}; EIMS: *m/z* 266 $[M]^+$ (96%), 251 $[M - Me]^+$ (100), 236 $[M - 2 Me]^+$ (52), 165 (84), 149 (39).

Elution with hexane - Et_2O (4 : 1) gave a mixture of **7** and **8** which were separated by preparative tlc (benzene - Et_2O 9 : 1). Pure **7** (18 mg) had ν_{max} 3480, 1612 and 1260 cm^{-1}; EIMS: *m/z* 280 [M]+ (76%), 265 (100), 250 (53), 237 (42). Pure **8** (16 mg) had ν_{max} 3475, 1614 and 1257 cm^{-1}; EIMS: *m/z* 280 (78%), 265 (100), 250 (44), 237 (51).

The acidic fraction was chromatographed on HCl washed silica gel. The fractions eluted with hexane - Et_2O (17 : 3) were rechromatographed on silica gel to give **4**, **5**, **12**, **14** and **16**. **4** (250 mg) (eluted with benzene - Et_2O 3 : 17) had ν_{max} 3350 and 1605 cm^{-1}; HPLC: *m/z* 266 (96%), 251 (100), 236 (45), 165 (80), 149 (50). **16**, eluted with benzene - Et_2O (1 : 19) was purified by preparative tlc (benzene - Et_2O 17 : 3) (5 mg): ν_{max} 3460, 1620 and 1140 cm^{-1}; EIMS: *m/z* 298 (100%), 283 (80), 255 (43). The mixture of **5**, **12** and **14**, eluted with benzene - Et_2O (1 : 19) was resolved by preparative reverse phase HPLC (RP-18, MeOH - H_2O 9 : 1) to give juncunone (**12**) (18 mg), **14** (16 mg) and **5** (125 mg). **14** had ν_{max}: 3340, 1705 and 1620 cm^{-1}; EIMS: *m/z* 268 (100%), 239 (51). **5** had ν_{max} 3340 and 1610 cm^{-1}; EIMS: *m/z* 266 (90%), 251 (100), 236 (40), 165 (80).

The fractions eluted with hexane - Et_2O (4 : 1), after chromatography on silica gel (hexane - Me_2CO 7 : 3) and reverse phase HPLC (RP-18, MeOH - H_2O (4: 1) gave juncusol (**1**) (16 mg) and **6** (130 mg). **6** had ν_{max} 1330 and 1605 cm^{-1}; EIMS: *m/z* 266 (85%), 251 (100), 236 (70).

The fractions eluted with benzene - Et_2O (7 : 3) were rechromatographed on silica gel. Benzene - Et_2O (19 : 1) gave effusol (**2**) (64 mg), purified by reverse phase HPLC (RP-18, MeOH - H_2O 3 : 1) and **13** (13 mg), purified by preparative tlc (hexane - Me_2CO 7 : 3). **13** had ν_{max} 3350, 1705, 1620 and1603 cm^{-1}; EIMS: *m/z* 282 (70%), 267 (100), 253 (45). Benzene - Et_2O (4 : 1) gave a mixture of **18** and **15** and a mixture of **9** and **10**. The first mixture was resolved by reverse phase HPLC (RP-18, MeOH - H_2O 3: 1) to give micrandol B (**18**) (7 mg) and **15** (40 mg). **15** had ν_{max} 3340 and 1605 cm^{-1}; EIMS: *m/z* 284 (100%), 266 (68). The latter mixture was separated by preparative tlc (benzene - Et_2O - AcOH 60 : 31 : 1) to give **9** (8 mg): ν_{max} 3520, 1690 and 1610 cm^{-1}; EIMS: *m/z* 280 (100%), 273 ((65), 235 (55) and **10** (10 mg): ν_{max} 3500, 1685, 1620 cm^{-1}; EIMS: *m/z* 280 (100), 273 (72), 235 (41).

The fractions eluted with hexane - Et_2O (3 : 2) gave after preparative tlc (benzene - Et_2O 1 : 3) **17** (22 mg): ν_{max} 3350 and 1610 cm^{-1}; EIMS *m/z* 284 (100), 266 (75).

Acknowledgments. This work was financially supported by Consiglio Nazionale delle Ricerche in the frame of the Progetto Finalizzato Chimica Fine. The Authors thanks Dr. A. De Giulio for the biological tests.

REFERENCES

1. Della Greca, M.; Fiorentino,A.; Mangoni,L.; Molinaro, A.; Monaco,P. and Previtera,L. *Tetrahedron Letters*, **1992**, *33*, 5257.
2. Shima,K.; Toyota,M. and Asakawa,J. *Phytochemistry*, **1991**, *30*, 3149.
3. Sarkar,H.; Zerezghi,M. and Bhattacharyya,J. *Phytochemistry*, **1988**, *27*, 3006.

4. Ferrigni,N.R.; Putnam,J.E.; Anderson,B.; Jacobsen,L.B.; Nichols,D.E.; Moore,D.S. and McLaughlin,J.L. *J. Nat. Prod.*, **1982**, *45*, 679.
5. Miles,D.H.; Randle,S.; Shakir,R and Atwood,J.L. *J. Org. Chem.*, **1981**, *46*, 2813.
6. De Alvarenga,M.A. and Gottlieb, O.R. *Phytochemistry*, **1974**, *13*, 1283.
7. Meyer,B.N.; Ferrigni,N.R.; Putnam,J.E.; Jacobsen,L.B.; Nichols,D.E. and McLaughlinJ.L. *Planta Med.*, **1982**, *45*, 31.
8. Cimino,G.; De Giulio,A.; De Rosa,S. and Di Marzo,V. *J. Nat. Prod.*, **1990**, *53*, 345.
9. Finney, D.J. Probit Analysis, 3rd ed. **1971**, Cambridge University Press, Cambridge.