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PHYTOCHEMISTRY

Bisquinolinone alkaloids from *Melicope ptelefolia*

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Abstract

The investigation of the leaves of *Melicope ptelefolia* (Rutaceae) afforded, besides *N*-methylflindersine, two new bisquinolinone alkaloids named melicobisquinolinone A and B. Their structures were established by MS and NMR spectroscopy, especially NOE and HMBC experiments. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Melicope ptelefolia; Rutaceae; Bisquinolinone alkaloids; Melicobisquinolinones A and B

1. Introduction

In continuation of our phytochemical studies on the Vietnamese medicinal plant *Melicope ptelefolia* (Champ. ex Benth.) Hartley [= *Evodia lepta* (Spreng.) Merr.], which afforded a series of 2,2-dimethyl-2*H*-1-benzopyrans (Kamperdick, Van, Sung, & Adam, 1997) and two benzopyran dimers (Kamperdick, Van, Sung, & Adam, 1998), we now report the isolation and structure elucidation of the two new bisquinolinone alkaloids, melicobisquinolinone A and B, from this plant.

2. Results and discussion

The *n*-hexane extract of the leaves upon silica gel chromatography yielded large amounts of an alkaloid of *m*/*z* 241 ($[M]^+$, $C_{15}H_{15}NO_2$), identified as *N*-methylflindersine (**1**) from its MS and NMR spectra (see Section 3).

In the same manner chromatography of the EtOAc extract afforded two minor alkaloids **2** and **3**. The HRMS of compound **2** shows the molecular ion as base peak at *m*/*z* 482.2188 with the elemental composition $C_{30}H_{30}N_2O_4$, corresponding exactly to two molecules of **1**. Other prominent peaks at *m*/*z* 241, 227 and 226 occur also in the MS of **1** representing there the $[M]^+$-, $[M–CH_2]^+$- and $[M–Me]^+$-ions. The ^{13}C NMR spectrum of **2** shows eight pairs of carbon signals which correspond in their chemical shifts to the quinolinone moiety of **1** (positions 5–10a). The presence of two quinolinone units is also confirmed by the C–H long-range correlations (Table 1), suggesting **2** as a dimer of **1**. Furthermore, the NMR spectra display a trisubstituted double bond, three aliphatic methine groups and four quaternary methyl groups. The sequence of the CH-groups follows from the $^3J_{HH}$ coupling constants [δ_H 4.53 (d, $J = 4.1$ Hz), 3.25 (dd, $J = 4.1$ and 1.9 Hz), 5.58 dd ($J = 9.5$ and 1.9 Hz), 5.17 (d, $J = 9.5$ Hz)]. From these data together with the $^1J_{CH}$ correlations from the HMQC experiment, the subunit -OCH–CH–CH–CH=C is established. The connection of this subunit with the two quinolinone moieties was deduced from the C–H long-range correlations (Table 1, Fig. 1) and establishes a six-cyclic constitution. The *cis*-junction of the two dihydropyran rings was obtained from the relatively small $^3J_{HH}$ coupling of 4.1 Hz between H-6a (δ 4.53) and H-14a (δ 3.25) and the NOE effect (Table 1) between these protons. According to this, the 6α-methyl group shows interactions to both H-6a and H-14a. The α-orientation of the isobutenyl side chain is revealed by the NOE effect between H-6a and the olefinic proton H-1′ (δ 5.17). The assignments of the (*E*)- and (*Z*)-2′-methyl groups were done according to the NOE inter-

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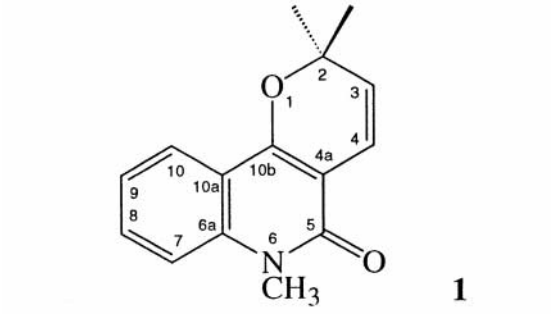

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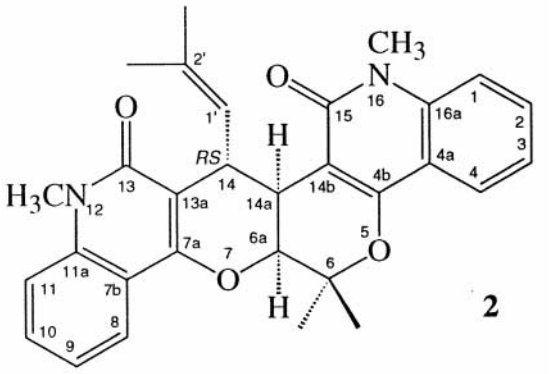

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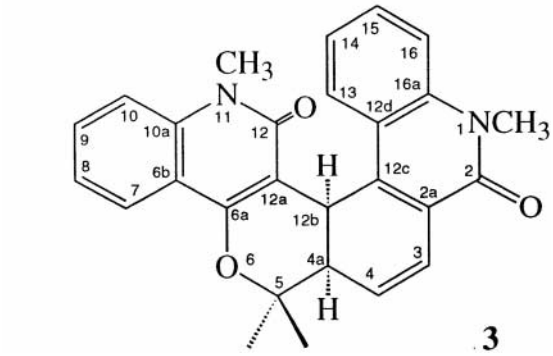

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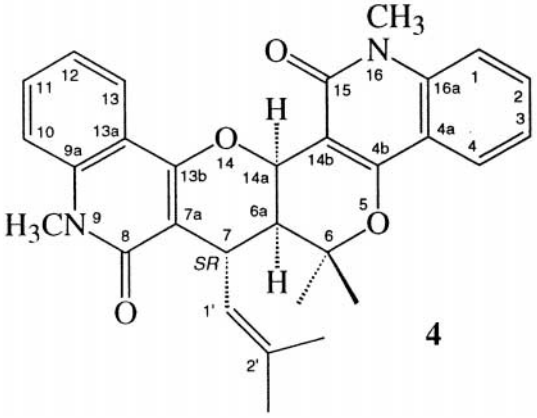

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actions between H-1 and the protons at δ 1.78 [2-Me (*E*)] as well as between H-14 (δ 5.58) and the protons at δ 2.25 [2-Me (*Z*)]. Further NOE effects of H-6a to both the 6α- and 6β-methyl groups, which are of equal size, indicate a half-chair or a twisted conformation for the dihydropyran ring with a staggered arrangement of the substituents and excludes a half-boat conformation. The second pyran ring is also supposed to take a half-chair or twisted conformation with a dihedral angle of about 90° between H-14 and H-14a (according to the Dreiding model), which explains the small $^3J_{HH}$ coupling of 1.9 Hz. Such a conformation is confirmed also by comparison with NMR data of paraensidimerine D (**4**), another dimer of **1**, which was isolated from *Euxylophora paraënsis* (Rutaceae). Especially the $^3J_{HH}$ couplings of **4** are very similar to those of **2**. From the X-ray analysis of **4**, the twisted conformation of the dihydropyran rings and a dihedral angle of 86.9° between H-6a and H-7 (which correspond to H-14 and H-14a in **2**) were obtained (Jurd, Wong, & Benson, 1982).

Paraensidimerine D (**4**) is supposed to be biosynthetically formed by a hetero Diels–Alder reaction of *N*-methylflindersine (**1**) with a C-prenylquinolinone precursor **1a** (Jurd et al., 1982; Ngadjui et al., 1989), arising formally from **1** by a retro Diels–Alder reaction. Thus, alkaloid **2** may be formed in a similar manner, however with a different arrangement of the educts (Fig. 2).

A striking feature in the NMR data of **2** is the strong deshielding of the aliphatic H-14 (δ 5.58) and the shielding of the connected carbon C-14 (δ 28.7). The two amide groups with a partially negative charged oxygen and a partially positive charged nitrogen cause electric fields. This C–H bond is situated close and parallel to both electric fields, which push the electrons from the proton H-14a towards the carbon.

The HRMS of alkaloid **3** gives the molecular ion at *m/z* 424.1768 indicating the elemental composition $C_{27}H_{24}N_2O_3$. The base peak at *m/z* 365 is formed by loss of -OC_3H_7. A fragment at *m/z* 226 [*N*-methylflindersine–Me]$^+$ suggests that **3** also contains a *N*-methylflindersine unit. As in **2** the NMR spectra of **3** shows two quinolinone units. Additionally, the NMR spectra exhibited one *cis*-disubstituted double bond (δ_H 6.06 and 6.98, J = 9.7 Hz, δ_C = 126.6 and 126.0), two aliphatic CH-groups (δ_H 2.77/δ_C 41.8 and δ_H 4.69/δ_C 30.0) and a quaternary carbon (δ_C 77.2) bearing one oxygen atom and two methyl groups (δ_C 25.8 and 25.5, δ_H 1.74 and 1.56). Analysis of the C–H long-range correlations from the HMBC experiment (Table 2 and Fig. 1) gives the connection of the two quinolinone units with these functional groups and the carbon assignments, indicating also for **3** a six-cyclic ring-constitution. The *cis*-junction of C-4a and C-12b follows from the NOE effect between H-4a (δ 2.77) and H-12b (δ 4.69). The differentiation of the 5α- and

Table 1. ^{13}C NMR and ^{1}H NMR spectral data of melicobisquinolinone A (**2**) in $CDCl_3$ (75/300 MHz)

	δ_C	δ_H (J in Hz)	C–H long-range correlations	NOE-effects
1	113.7[a]	7.22 [a] m	H-3 (w)	
2	130.5	7.45 m	H-4 (w)	
3	121.2	7.16[b] m	H-1	
4	123.3	7.93 dd (8.1/1.5)	H-2	
4a	115.8[b]	–	H-1, H-3	
4b	156.3	–		
6	77.9	–	H-6a, 6α-Me, 6β-Me	
6a	73.7	4.53 d (4.1)	H-14, 6α-Me, 6β-Me	H-14a, H-1′, 6β-Me
7a	153.9	–	H-14	
7b	116.0[b]	–	H-9, H-11	
8	122.6	7.89 dd (8.1/1.5)	H-10	
9	121.2	7.13[b] m	H-11	
10	129.9	7.45 m	H-8	
11	113.6[a]	7.21[a]	H-10	
11a	138.8	–	H-8, H-10, 12-Me	
13	162.54[c]	–	H-14 (w), 12-Me	
13a	108.5	–	H-14	
14	28.7	5.58 dd (9.5/1.9)		H-14a, 2′-Me (*Z*)
14a	34.5	3.25 dd (4.1/1.9)		H-6a, H-14, H-1′, 6α-Me
14b	104.0	–	H-6a, H-14	
15	162.45[c]	–	16-Me	
16a	139.1	–	H-2, H-4, 16-Me	
1′	126.5	5.17 dm (9.5)	H-14, 2′-Me (E), 2′-Me (Z)	H-6a, 2′-Me (*E*)
2′	135.5	–	H-14, 2′-Me (E), 2′-Me (Z)	
6α-Me	22.8	1.38 s	6β-Me	H-6a, H-14a, 6β-Me
6β-Me	25.2	1.93 s	6α-Me	H-8, H-6a, 6α-Me
12-Me	29.24[d]	3.61[c] s		
16-Me	29.17[d]	3.60[c] s		
2′-Me (*E*)	26.1	1.78 d (1.1)	2′-Me (*Z*)	H-1′, 2′-Me (*Z*)
2′-Me (*Z*)	18.8	2.25 d (1.1)	2′Me (*E*)	H-14, H-14a (w)

[a],[b],[c],[d]Assignments interchangeable.
w = weak.

the 5β-methyl group is enabled by the NOE interaction between H-12b (δ 4.69, α) and the methyl protons at δ 1.56, which thus belongs to the α-methyl group. Accordingly, the 5β-methyl group at δ 1.74 shows an NOE interaction to H-4 (δ 6.06). Like in **2**, the nearly equal-sized NOE effects of both methyl groups at C-5 (δ_H 1.56 and 1.74) indicate the expected half-chair or twisted conformation of the dihydropyran ring. The residual NOE effects (Table 2) confirm the configuration of **3**.

Because both alkaloids **2** and **3** show no Cotton effect in the CD spectra, they are suggested to be racemic.

N-methylflindersine is a characteristic constituent of plants from the Rutaceae and Meliaceae family. It is known to possess insect growth inhibitory, antifeedant and fungistatic activities (Hegnauer, 1990). The alkaloids **1**–**3** were tested for fungitoxic activity against *Cladosporium cucumerinum*. The lowest amount necessary to inhibit mycel growth, was 12.5 nmol of **1** and 3 nmol of **3**. Alkaloid **2** showed no activity, which reflects, compared with the found high activity of **3**, a dramatic structural influence of both dimer types.

3. Experimental

EI-MS: AMD 402, 70 eV. NMR: 1D: Varian Gemini 300, 2D: Varian Unity 500. CC: silica gel 60, 70–200 and 230–400 mesh ASTM (Merck); TLC: precoated silica gel plates 60 F_{254}, thickness 1 mm (Merck).

3.1. Plant material

Leaves and branches of *Melicope ptelefolia* were collected in Lao cai, North Vietnam, in June 1994 and identified by Dr T.D. Dai. A voucher specimen (No. 382) is deposited in the Institute of Ecology and Natural Resources, National Centre for Natural Science and Technology, Hanoi, Vietnam.

3.2. Extraction and isolation

Dried leaves (500 g) were extracted 3× with 80% aq. MeOH at room temp. and the organic solvent removed under red. pres. The aq. residue was extracted 3× with *n*-hexane and 3× with EtOAc, giving 17.3 g

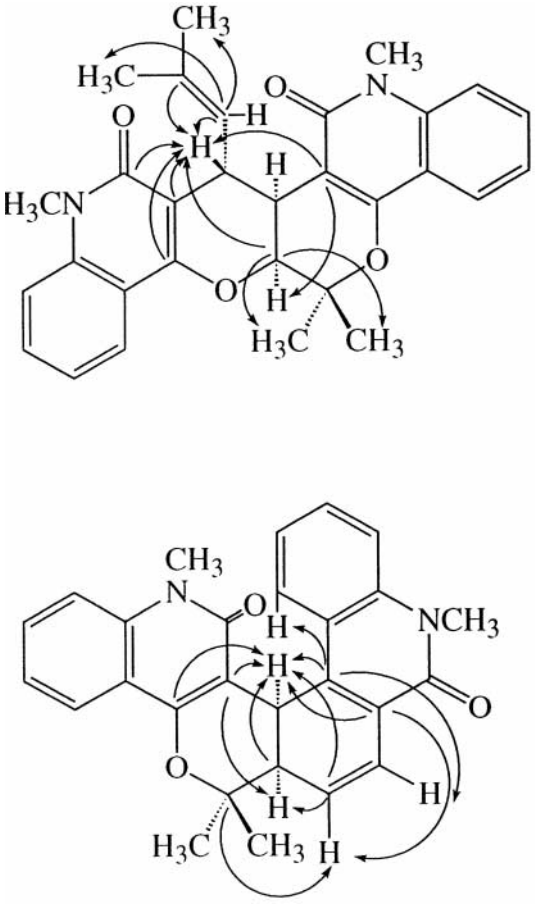

Fig. 1.

n-hexane extract and 15.8 g EtOAc extract. The *n*-hexane extract was sepd by chromatography on silica gel (200 g, 70–200 mesh) with increasing amounts of EtOAc in *n*-hexane as eluent (2–100% EtOAc, 237 frs, each 20 ml). Frs 205–236 (2.43 g) afforded 1.35 g of **1** by chromatography on silica gel 60 (230–400 mesh) using *n*-hexane–EtOAc (1:1).

The EtOAc extract was fractionated on silica gel 60 (250 g, 70–200 mesh) with an eluent of increasing polarity (10–100% $CHCl_3$ in *n*-hexane, followed by 10–50% MeOH in $CHCl_3$) giving 400 frs with 20 ml. Frs 82–112 (1.51 g) yielded upon further chromatography 0.60 g of **1**. Frs 112–121 (1.59 g) were chromatographed by CC on silica gel 60 (150 g, 230–400 mesh) using *n*-hexane–EtOAc (4:1). Frs 22–30 (170 mg) were further purified by CC on silica gel 60 (230–400 mesh) eluting with *n*-hexane–EtOAc (1:1) followed by preparative TLC using *n*-hexane–$CHCl_3$–diethylamin (1:4:1) to afford 4.9 mg of **3**. Preparative TLC of frs 31–40 (30 mg) using *n*-hexane–EtOAc (1:9) yielded 7.2 mg of **2**.

The bioassay with *Cladosporium cucumerinum* was done according to Gottstein, Groß, and Lehmann (1984).

3.2.1. *N-Methylflindersine* (**1**)

Crystals, m.p. 82–84. UV λ_{max}^{MeOH} nm (log ε): 348 (4.17), 226 (4.62). IR ν_{max}^{KBr} (cm^{-1}): 3055, 2971, 2934, 1642, 1623, 1569, 1507, 1461, 1417, 1359, 1325, 1148, 1123, 1091, 895, 747, 720. MS *m*/*z* (rel. int.): 241 $[M]^+$ (13), 227 $[M–CH_2]^+$ (15), 226 $[M–Me]^+$ (100), 211 $[M–2Me]^+$ (1.4), 200 (1.8), 183 (3.2), 113 (3.7). ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.97 (1 H, dd, J = 8.2 and 1.5 Hz, H-10), 7.55 (1 H, ddd, J = 8.6, 7.1 and 1.5 Hz,
H-8), 7.32 (1 H, d, J = 8.6 Hz, H-7), 7.23 (1 H, ddd, J = 8.2, 7.1 and 1.0 Hz, H-9), 6.76 (1 H, d, J = 9.8 Hz, H-4), 5.54 (1 H, d, J = 9.8Hz, H-3), 3.70 (3 H, s, N–Me), 1.52 (6 H, s, 2-Me_2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 161.0 (C-5), 155.2 (C-10b), 139.4 (C-6a), 130.8 (C-8), 126.3 (C-3), 123.1 (C-10), 121.7 (C-9), 118.0 (C-4), 116.1 (C-10a), 114.0 (C-7), 105.9 (C-4a), 78.7 (C-2), 29.2 (NMe), 28.2 (2-Me_2). The proton shifts are in agreement with reference data (Hifnawy, Vaquette, Sévenet, Pousset, & Cavé, 1977). As no carbon shifts of **1** could be found in the literature, these data are added here. Carbons were assigned by comparison with **2** and **3**.

3.2.2. *Melicobisquinolinone A* (**2**)

Amorphous. UV λ_{max}^{MeOH} nm (log ε): 318 (4.13), 274 (4.14), 226 (4.92). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3002, 2929, 2856, 1646, 1625, 1595, 1577, 1503, 1465, 1389, 1331, 1190, 1164, 1120, 1094. MS *m*/*z* (rel. int.): 482 $[M]^+$ (100), 467 $[M–Me]^+$ (5), 439 (23), 427 (33), 308 (5), 294 (6), 264 (6), 252 (15), 242 [*N*-methylflindersine + H]$^+$ (51), 241 [*N*-methylflindersine]$^+$ (65), 227 [*N*-methylflinder-

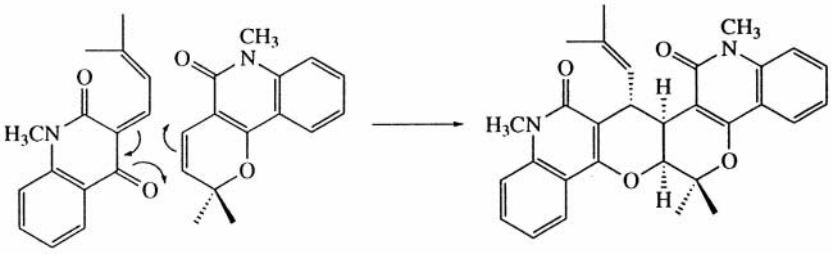

Fig. 2.

Table 2. ^{13}C NMR and ^{1}H NMR spectral data of melicobisquinolinone B (**3**) in $CDCl_3$ (75/300 MHz)

	δ_C	δ_H (J in Hz)	CH long-range correlation	NOE-effects
2	160.0	–	1-Me	
2a	123.3	–	H-4, H-12b	
3	126.0	6.98 dd (9.7/2.7)	H-4	H-4
4	126.62[a]	6.06 d (9.7)	H-4a (w), H-12b	H-3, H-4a, 5β-Me
4a	41.8	2.77 dd (5.5/2.7)	H-3 (w), H-4, H-12b, 5α-Me, 5β-Me	H-4, H-12b, 5α-Me, 5β-Me
5	77.2	–	5α-Me, 5β-Me, H-4 (w)	
6a	157.6	–	H-7, H-12b,	
6b	116.0	–	H-8, H-10	
7	123.2	7.99 dd (8.6/1.5)	H-9	H-8
8	121.3	7.20 t (7.3)	H-10	
9	130.6	7.51 m	H-7	
10	113.6	7.21 d (7.6)	H-8	
10a	138.9	–	H-7, H-9, 11-Me	
12	161.9	–	11-Me	
12a	104.2	–	H-4a (w), H-12b	
12b	30.0	4.69 d (5.5)	H-4 (w)	H-4a, H-13, 5α-Me
12c	142.0	–	H-3 (w), H-12b, H-13	
12d	123.0	–	H-12b, H-14, H-16	
13	126.56[a]	8.28 d (7.9)	H-15	H-12b, H-14
14	121.6	7.35 m	H-16	
15	129.1	7.54 m	H-13, H-16 (w)	
16	114.6	7.40 d (7.9)	H-14 (w)	
16a	138.5	–	H-13, H-15, 1-Me	
1-Me	29.8	3.75 s		H-16
5α-Me	25.5	1.56 s	5β-Me	H-4a, H-7 (w), H-12b, 5β-Me
5β-Me	25.8	1.74 s	5α-Me	H-4, H-4a, H-7 (w), 5α-Me
11-Me	29.4	3.43 s		H-10

[a]Assignment interchangeable.
w = weak.

sine–CH_2]$^+$ (21), 226 [*N*-methylflindersine–Me]$^+$ (97), 176 (8), 134 (11). HRMS 482.2188 [M]$^+$ ($C_{30}H_{30}N_2O_4$ requires 482.2206).

3.2.3. *Melicobisquinolinone B* (**3**)

Amorphous. UV λ_{max}^{MeOH} nm (log ε): 322 (4.08), 229 (4.80). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3000, 2929, 2855, 1642, 1620, 1592, 1502, 1464, 1385, 1328, 1158, 1119, 1015. MS *m*/*z* (rel. int.): 424 [M]$^+$ (43), 366 [M–C_3H_6O]$^+$ (43), 365 [M–C_3H_7O]$^+$ (100), 351 (23), 349 (16), 337 (15), 226 [*N*-methylflindersine–Me]$^+$ (11), 192 (15). HRMS 424.1768 [M]$^+$ ($C_{27}H_{24}N_2O_3$ requires 424.1787).

Acknowledgements

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References

Gottstein, D., Groß, D., & Lehmann, H. (1984). *Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete, 30,* 620.

Hegnauer, R., Chemotaxonomie der Pflanzen, Band IX. Birkhäuser, Basel, 1990, pp. 38, 449.

Hifnawy, M. S., Vaquette, J., Sévenet, T., Pousset, J. -L., & Cavé, A. (1977). *Phytochemistry, 16,* 1035.

Jurd, L., Wong, R. Y., & Benson, M. (1982). *Australian Journal of Chemistry, 35,* 2505.

Kamperdick, C., Van, N. H., Sung, T. V., & Adam, G. (1997). *Phytochemistry, 45,* 1049.

Kamperdick, C., Van, N.H., Sung, T.V., & Adam, G. (1998) Phytochemistry, *48*, 1055.

Ngadjui, B. T., Ayafor, J. F., Mitaku, S., Skaltsounis, A. -L., Tillequin, F., & Koch, M. (1989). *Journal of Natural Products, 52,* 300.