Isolation of Bioactive and Other Oxoaporphine Alkaloids from Two Annonaceous Plants, Xylopia aethiopica and Miliusa cf. banacea

George G. Harrigan, A. A. Leslie Gunatilaka, David G. I. Kingston, George W. Chan, and Randall K. Johnson

J. Nat. Prod., **1994**, 57 (1), 68-73• DOI: 10.1021/np50103a009 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 1, pp. 68-73, January 1994

ISOLATION OF BIOACTIVE AND OTHER OXOAPORPHINE ALKALOIDS FROM TWO ANNONACEOUS PLANTS, *XYLOPIA AETHIOPICA* AND *MILIUSA* CF. *BANACEA*

GEORGE G. HARRIGAN, A.A. LESLIE GUNATILAKA, DAVID G.I. KINGSTON,*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

GEORGE W. CHAN, and RANDALL K. JOHNSON*

Research and Development, SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939

ABSTRACT.—The oxoaporphine alkaloids oxophoebine [**1**] and liriodenine [**2**] have been isolated from *Xylopia aethiopica* (Annonaceae). Both showed selective toxicity against DNA repair and recombination deficient mutants of the yeast *Saccharomyces cerevisiae*. Three related but inactive compounds, oxoglaucine [**3**], *O*-methylmoschatoline [**4**], and lysicamine [**5**], were also isolated from this plant. Selective toxicity was also observed for 10-methoxyliriodenine (lauterine) [**6**] and 10-hydroxyliriodenine [**7**], two oxoaporphine alkaloids isolated from *Miliusa* cf. *banacea* (Annonaceae). The structure of 10-hydroxyliriodenine [**7**], a novel oxoaporphine, was determined by spectroscopic methods and chemical conversion to compound **6**. The role of the bioactive oxoaporphine alkaloids as DNA topoisomerase inhibitors is discussed.

Mechanism-based bioassays involving DNA repair or recombination deficient yeast mutants have been increasingly utilized in attempts to monitor the isolation of natural products with selective anti-cancer activity (1–7). This approach has now been applied to an investigation of two Annonaceous plants, the West African forest tree *Xylopia aethiopica* (Dunal) A. Rich and the Indopacific shrub *Miliusa* cf. *banacea* Finet and Gagnep.

Previous phytochemical studies on *X. aethiopica* have led to the isolation of kaurane and kaur-16-ene diterpenes from the fruits (8–10) and kaur-16-ene, kolavane, and trachylobane-type diterpenes from the stem bark (11). The genus *Miliusa* consists of approximately 40 species indigenous to Indonesia and Australia (12) and does not appear to have been previously subjected to phytochemical investigation.

RESULTS AND DISCUSSION

Three major DNA repair pathways have been defined in yeast and are known as the RAD 3, RAD 6, and RAD 52 pathways. The RAD 3 pathway is associated with excision, the RAD 6 pathway is the error-prone recombinational pathway, and RAD 52 is associated with the repair of double strand breaks and meiotic recombination (13). Any plant extract that inhibits yeast mutants lacking one or more of these pathways to a greater extent than it inhibits the wild-type strain (*rad*$^+$) should contain agents that induce DNA damage. A mutant RAD 52 repair-deficient strain, *rad 52. top 1*, with an additional deletion of the DNA topoisomerase I gene, can also detect agents that produce DNA damage specifically by interacting with DNA topoisomerase (14). Thus, an agent that exhibits greater activity towards the *rad 52. top 1* strain than to the *rad 52* strain, with a differential of at least three, most probably mediates its activity through inhibition of DNA topoisomerase II. Conversely, greater toxicity towards *rad 52* implies the presence of a DNA topoisomerase I inhibitor.

The MeOH extract of the aerial parts of *X. aethiopica* showed good activity against *rad 52* and *rad 6* mutants but was inactive against the *rad*$^+$ strain (Table 1). The MeCOEt extract of this plant also showed good activity against *rad 52* and *rad 52. top 1* mutants,

TABLE 1. Bioactivities of *Xylopia aethiopica* and *Miliusa* cf. *banacea* Extracts and Alkaloids.

Extract or Compound	*Saccharomyces cerevisiae* strain[a]			
	rad 52	*rad 52. top 1*	*rad 6*	*rad*$^+$
X. aethiopica (MeOH)	475	440	2165	>8000
X. aethiopica (MeCOEt)	528	934	>8000	>8000
Oxophoebine [**1**]	6.4[c]	2.6[d]	4.7	>500
Liriodenine [**2**]	16.7	NT	15.0 at 500[b]	500
Oxoglaucine [**3**]	>500	>500	>500	>500
O-Methyl-moschatoline [**4**]	>500	>500	>500	NT
Lysicamine [**5**]	300	390	>500	NT
Miliusa cf. *banacea* (MeCOEt)	>8000	2000	NT	>8000
Lauterine [**6**]	214	113	NT	NT
10-Hydroxyliriodenine [**7**]	295	72	NT	NT
Camptothecin	0.6	>20	8.7	NT
Streptonigrin	0.4	<0.4	2.4	NT

[a]Results are expressed as IC_{12} (μg/ml) and are the result of a single determination unless specified otherwise. NT=not tested.

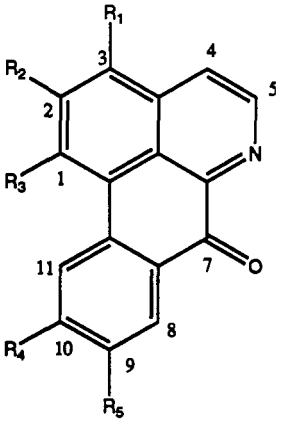
[b]Zone of inhibition in mm at the given conc. (μg/ml).

[c]Average of 3 determinations.

[d]Average of 2 determinations.

but was inactive against both *rad 6* and *rad*$^+$ strains (Table 1). The MeCOEt extract of the roots of *M.* cf. *banacea* showed activity against the *rad 52. top 1* mutant but was inactive against both the *rad 52* and *rad*$^+$ strains (Table 1).

Bioassay-guided fractionation involving liquid-liquid partition, Sephadex LH-20 adsorption chromatography, and Si gel chromatography of the MeOH and MeCOEt extracts of *X. aethiopica* led to the isolation of oxophoebine [**1**] and liriodenine [**2**], oxoaporphine alkaloids with selective activity against *rad 52* and *rad 6* mutants. Oxophoebine [**1**] was also toxic to the *rad 52. top 1* mutant (Table 1). Compound **1** was



	R_1	R_2	R_3	R_4	R_5
1	OMe	OMe	OMe	-O-CH_2-O-	
2	H	-O-CH_2-O-		H	H
3	H	OMe	OMe	OMe	OMe
4	OMe	OMe	OMe	H	H
5	H	OMe	OMe	H	H
6	H	-O-CH_2-O-		OMe	H
7	H	-O-CH_2-O-		OH	H

identified by comparison of ^{1}H-nmr, uv, and ms data with literature values (15,16). The identity of compound **2** was established by comparison of uv, ir, and ms data with literature values (16) and confirmed by ^{1}H-nmr spectroscopy.

A third isolated alkaloid, oxoglaucine [**3**], was identified by comparison of ^{1}H-nmr, uv, and ms data with literature values (16). The ir, ms, uv, and ^{1}H-nmr spectra of a fourth alkaloid correlated well with those reported for *O*-methylmoschatoline [**4**] (16,17), and the ms, uv, and ^{1}H-nmr data for a fifth alkaloid were consistent with those for lysicamine [**5**] (16). The alkaloids **3** and **4** when tested were found to be inactive in the *rad 52, rad 52. top 1* and *rad 6* assays, whereas alkaloid **5** exhibited weak activity against the *rad 52* and *rad 52. top 1* strains.

Bioassay-guided fractionation of the MeCOEt extract of *M.* cf. *banacea*, involving repeated Si gel and reversed-phase chromatography, yielded 10-methoxyliriodenine [**6**] and the novel 10-hydroxyliriodenine [**7**], oxoaporphine alkaloids with selective activity towards the *rad 52* and *rad 52. top 1* mutants (Table 1). The structure of compound **6** was established by comparison of ^{1}H-nmr, uv, and ms data with literature values (16,18). Spectral analyses of compound **7** showed that it was closely related to 10-methoxyliriodenine [**6**]. The ^{1}H-nmr spectrum of **7** matched that of 10-methoxyliriodenine [**6**] except for the absence of a MeO signal. Compound **7** also showed a base shift in its uv spectrum, and one exchangeable proton was observed in its ^{1}H-nmr spectrum. Upon treatment with CH_2N_2, compound **7** was converted to compound **6**, thereby clearly demonstrating a structure with an OH substituent at the 10 position rather than the 9 position as in oxoanolobine (19).

Lauterine [**6**] and 10-hydroxy-liriodenine [**7**] showed greater toxicity towards the *rad 52. top 1* mutant than to *rad 52* (Table 1). These two alkaloids were further evaluated for inhibition of purified mammalian DNA topoisomerase II (20), and, as predicted, were shown to be active (Table 2). 10-Hydroxyliriodenine [**7**] was about twice as active as lauterine [**6**], consistent with the larger differential between its activity towards the *rad 52. top 1* and *rad 52* mutants.

It is also interesting to note the activity conferred by the presence of a methylenedioxy group in the oxoaporphine alkaloids. Thus, oxophoebine [**1**], which contains a C-9–C-10 methylenedioxy group, is orders of magnitude more active than the related alkaloids oxoglaucine [**3**] and *O*-methylmoschatoline [**4**], which lack the group. Similarily, liriodenine [**2**], which has a methylenedioxy group at C-1–C-2, is many orders of magnitude more active than lysicamine [**5**], which is identical except that it has MeO groups at C-1 and C-2. By the same token, lauterine [**6**] and 10-hydroxyliriodenine [**7**], which are significantly toxic to the *rad 52. top 1* mutant and inhibit DNA topoisomerase II activity, also possess the methylenedioxy function at C-1–C-2. Interestingly, substitution at the 10-position of liriodenine, as in compounds **6** and **7**, reduces its toxicity to the *rad 52* mutant (Table 1).

Liriodenine [**2**] has been shown previously to be cytotoxic in the KB system (21), and cytotoxic activity has also been reported for oxoglaucine [**3**] (22). Lauterine [**6**] was examined only spectroscopically in the literature, and no previous biological activity has been reported for it.

TABLE 2. Bioactivity of *Miliusa* cf. *banacea* Alkaloids.

Compound	Inhibition of Topoisomerase II Unknotting Activity in vitro[a]
Lauterine [**6**]	25.0
10-Hydroxyliriodenine [**7**]	12.5

[a]Results expressed as IC_{50} (μg/ml).

In conclusion, certain oxoaporphinoid alkaloids, particularly those with methylenedioxy substituents, may represent a novel class of DNA topoisomerase inhibitors.

EXPERIMENTAL

PLANT MATERIAL.—The twigs, leaves, and stem bark of *X. aethiopica* were collected by A.A. Enti (Forestry Enterprises) in the rain forest in the Bobiri Reserve at Kumasi (Ghana) in January 1990. Voucher specimens are kept by A.A. Enti (Ghana) and Biotic (UK). Extracts for screening were prepared by sequential solvent extraction with hexane, MeCOEt, and MeOH. *M* cf. *banacea* was collected in Thailand in December 1979 and identified by Dr. R.J. Spjut, World Botanical Associates, Laurel, Maryland. A voucher specimen is located at the US National Arboretum, Washington, DC. Extracts for screening were prepared from dry pulverized roots of the plant through sequential solvent extraction with hexane, MeCOEt, and MeOH.

GENERAL EXPERIMENTAL METHODS.—Uv spectra were obtained in MeOH on a Beckman DU-50 spectrophotometer. Ir spectra were obtained on a Perkin-Elmer Model 1600 spectrometer. Ms for compounds **1–5** were taken at 70 eV on a VG 7070 E-HF instrument with direct probe application. Low resolution ms of compounds **6** and **7** were obtained on a Finnigan MAT 4610 mass analyzer with direct probe application. The ^{1}H-nmr spectra for compounds **1–5** were recorded on a Bruker 270 spectrometer at 270 MHz with TMS as internal standard and $CDCl_3$ as solvent. For compounds **6** and **7**, ^{1}H-nmr spectra were obtained on a Bruker 400 spectrometer at 400 MHz with TMS as internal standard and CF_3COOD as solvent.

BIOLOGICAL SCREENING PROCEDURES.—The experimental methods utilized in the screening procedure have been described elsewhere (5). The IC_{12} values refer to the concentration in μg/ml required to produce a zone of inhibition of 12 mm diameter around a 100 μl well during a 48 h incubation period at 37°.

DNA TOPOISOMERASE II UNKNOTTING INHIBITION ASSAYS.—These assays were carried out as described previously (20).

EXTRACTION AND ISOLATION.—A MeOH (122.3 g) and a MeCOEt extract (44.9 g) of *X. aethiopica* were separately partitioned between *n*-BuOH and H_2O (all solvents were hplc grade), the *n*-BuOH fraction further partitioned between hexane and 80% aqueous MeOH, and the aqueous MeOH fraction then subjected to liquid-liquid partition between $CHCl_3$ and 60% aqueous MeOH. The $CHCl_3$ fraction was subjected to gel permeation/adsorption chromatography on Sephadex LH-20 (ca. 30 g, Sigma) (23) eluting initially with hexane-CH_2Cl_2 (1:1), followed by CH_2Cl_2, CH_2Cl_2-Me_2CO (1:1), and finally MeOH. This chromatographic step was repeated on the hexane/CH_2Cl_2 fraction obtained from the MeOH extract this time eluting with hexane-CH_2Cl_2 (4:1), followed again by CH_2Cl_2, CH_2Cl_2-Me_2CO (1:1), and finally MeOH. The hexane-CH_2Cl_2 (4:1) fraction was subjected to rotary centrifugal chromatography on Si gel GF_{254} (Analtech, 2 mm thickness) eluting initially with hexane-CH_2Cl_2 (2:3) followed by solutions containing progressively increasing amounts of CH_2Cl_2, followed by CH_2Cl_2 solutions containing progressively increasing amounts of iPrOH. A fraction was collected between 2–5% iPrOH in CH_2Cl_2, and preparative tlc separation on Si gel GF_{254} (Analtech, 0.5 mm thickness) was then performed on this fraction using CH_2Cl_2-iPrOH (19:1) as mobile phase. Three bands, R_f 0.67 (**4**, 25 mg), R_f 0.63 (**1**, 6 mg), and R_f 0.57 (**5**, 20 mg), were collected, and non-alkaloidal impurities were removed by acid-base extraction. Gel permeation/adsorption chromatography on Sephadex LH-20 (ca. 30 g) was also performed on the hexane-CH_2Cl_2 (1:1) fraction obtained from the MeCOEt extract; elution was initiated with hexane-CH_2Cl_2 (4:1) followed by hexane-CH_2Cl_2 (1:1). The hexane-CH_2Cl_2 (1:1) fraction was subjected to Si gel (230–400 mesh, ca. 10 g, EM Science) chromatography eluting initially with CH_2Cl_2 followed by CH_2Cl_2 solutions containing progressively increasing amounts of iPrOH. The fraction collected between 2–5% iPrOH in CH_2Cl_2 was subjected to preparative tlc as above, yielding compound **3** (R_f 0.18, 7.1 mg). A band was also collected at R_f 0.30 and resubjected to preparative tlc using CH_2Cl_2-iPrOH (99:1) (triple elution) as mobile phase to yield compound **2** (1 mg).

The MeCOEt extract (2.8 g) of *M. cf. banacea* was chromatographed on Si gel using CH_2Cl_2 solutions containing progressively increasing amounts of MeOH. The fraction collected between 2–4% MeOH was subjected to reversed-phase hplc (Whatman, Partisil ODS) using an MeCN gradient in 0.2% aqueous TFA. Both compounds **6** and **7** were eluted with 30% MeCN. Fractions containing only compound **7** (hplc Rt 9.8 min, Beckman Ultrasphere ODS, 150×4.6 mm, 20–80% MeCN in 0.2% aqueous TFA, uv 254 nm, 1.5 ml/min) were combined and lyophilized to a brown residue (5.9 mg), which was subsequently subjected to preparative tlc on Si gel (Merck Kieselgur, 0.25 mm thickness) using CH_2Cl_2-MeOH (20:1) as mobile phase to yield pure compound **7** (2.5 mg). The MeCN fractions containing only compound **6** (hplc Rt 11.2

min, Beckman Ultrasphere ODS, 150×4.6 mm, 20–80% MeCN in 0.2% aqueous TFA, uv 254 nm, 1.5 ml/min) were combined and lyophilized to an orange-brown residue (4.7 mg), which was subjected to preparative tlc on Si gel (Merck Kieselgur, 0.25 mm thickness) using CH_2Cl_2-MeOH (20:1) as mobile phase to give pure compound **6** (1.5 mg).

Oxophoebine [**1**].—Red amorphous solid: uv λ max (MeOH) 211, 240, 274, 319 sh, 379, 477 nm; ^{1}H nmr ($CDCl_3$, 270 MHz) δ 8.96 (1H, d, J=5.0 Hz, H-5), 8.61 (1H, s, H-11), 8.19 (1H, d, J=5.0 Hz, H-4), 7.99 (1H, s, H-8), 6.14 (2H, s, O-CH_2-O), 4.18 (3H, s, OMe), 4.09 (3H, s, OMe), 4.07 (3H, s, OMe).

Liriodenine [**2**].—Yellow amorphous powder: uv λ max (MeOH) 219 sh, 246, 271, 307, 410 nm; ^{1}H nmr ($CDCl_3$, 270 MHz) δ 8.90 (1H, d, J=5.3 Hz, H-5), 8.67 (1H, d, J=8.3 Hz, H-8/11), 8.60 (1H, d, J=8.3 Hz, H-8/11), 7.78 (1H, d, J=5.3 Hz, H-4), 7.76 (1H, t, J=8.3 Hz, H-9/10), 7.60 (1H, t, J=8.3 Hz, H-9/10), 7.21 (1H, s, H-3), 6.38 (2H, s, O-CH_2-O).

Oxoglaucine [**3**].—Orange amorphous solid: uv λ max (MeOH) 240, 270, 288 sh, 353 nm; ^{1}H nmr ($CDCl_3$, 270 MHz) δ 8.92 (1H, d, J=5.3 Hz, H-5), 8.83 (1H, s, H-11), 8.03 (1H, s, H-8), 7.79 (1H, d, J=5.3 Hz, H-4), 7.21 (1H, s, H-3), 4.11 (3H, s, OMe), 4.08 (6H, s, OMe×2), 4.03 (3H, s, OMe).

O-*Methylmoschatoline* [**4**].—Orange amorphous solid: uv λ max (MeOH) 212, 228, 271, 314 sh, 434 nm; ^{1}H nmr ($CDCl_3$, 270 MHz) δ 9.11 (1H, dd, J=7.9, 0.7 Hz, H-11), 8.97 (1H, d, J=5.4 Hz, H-5), 8.58 (1H, dd, J=7.9, 1.6 Hz, H-8), 8.22 (1H, d, J=5.4 Hz, H-4), 7.74 (1H, dt, J=7.9, 1.6 Hz, H-10), 7.56 (1H, dt, J=7.9, 0.7 Hz, H-9), 4.17 (3H, s, OMe), 4.11 (3H, s, OMe), 4.08 (3H, s, OMe).

Lysicamine [**5**].—Yellow amorphous powder: uv λ max (MeOH) 230, 267, 314, 372 sh, 388 nm; ^{1}H nmr ($CDCl_3$, 270 MHz) δ 9.18 (1H, dd, J=7.8, 0.9 Hz, H-11), 8.91 (1H, d, J=5.1 Hz, H-5), 8.59 (1H, dd, J=7.8, 1.5 Hz, H-8), 7.81 (1H, d, J=5.1 Hz, H-4), 7.78 (1H, dt, J=7.8, 1.5 Hz, H-10), 7.61 (1H, dt, J=7.8, 0.9 Hz, H-9), 7.23 (1H, s, H-3), 4.11 (3H, s, OMe), 4.02 (3H, s, OMe).

10-Methoxyliriodenine [**6**].—Yellow amorphous solid: uv λ max (MeOH) 247, 309, 343, 405 nm, no change with addition of base; eims *m/z* (rel. int.) $[M]^+$ 305 (100) for $C_{18}H_{11}NO_4$, 276 (10), 234 (30), 206 (43), 176 (20); ^{1}H nmr (CF_3COOD, 400 MHz) δ 8.78 (1H, d, J=6.5 Hz, H-5), 8.65 (1H, d, J=8.5 Hz, H-8), 8.47 (1H, d, J=6.5 Hz, H-4), 8.10 (1H, d, J=2.5 Hz, H-11), 7.63 (1H, s, H-3), 7.45 (1H, dd, J=8.5, 2.5 Hz, H-9), 6.70 (2H, s, O-CH_2-O), 4.20 (3H, s, OMe).

10-Hydroxyliriodenine [**7**].—Orange amorphous solid: uv λ max (MeOH) 210, 246, 269, 354, 420 nm; uv λ max (MeOH+NaOH) 212, 256, 286, 330, 373, 473 nm; cims *m/z* (rel. int.) $[MH]^+$ 292 (100) for $C_{17}H_{10}NO_4$, 264 (42), 234 (21), 179 (20); ^{1}H nmr (CF_3COOD, 400 MHz) δ 8.79 (1H, d, J=6.0 Hz, H-5), 8.60 (1H, d, J=8.0 Hz, H-8), 8.45 (1H, d, J=6.0 Hz, H-4), 8.08 (1H, d, J=3.0 Hz, H-11), 7.61 (1H, s, H-3), 7.45 (1H, dd, J=8.0, 3.0 Hz, H-9), 6.69 (2H, s, O-CH_2-O).

CH_2N_2 treatment of compound **7**.—An orange-colored solution of compound **7** (1.0 mg) in 0.2 ml of absolute MeOH was treated with 0.4 ml of a 0.3 M ethereal solution of CH_2N_2. The residue was purified by preparative tlc on Si gel (Merck Kieselgur) using CH_2Cl_2-MeOH (20:1) as mobile phase to give a methylation product identical, spectroscopically and chromatographically, to compound **6**.

ACKNOWLEDGMENTS

This work was supported by a National Cooperative Drug Discovery Group grant awarded to the University of Virginia (I UO1 CA 50771) (Dr. S.M. Hecht, Principal Investigator). We thank Mr. Kim Harich (VPI and SU) for high resolution ms and Ms. Nina Baj (VPI and SU) for expert technical assistance. We also thank Forestry Enterprises of Ghana through Biotics Limited (UK) for the supply of *X. aethiopica* plant material, and Dr. R.J. Spjut, World Botanical Associates, for identification of *Miliusa* cf. *banacea*.

LITERATURE CITED

1. L.J. Hanka, in: "Advances in Applied Microbiology." Ed. by D. Perlman, Academic Press, New York, 1972, Vol. 15, pp. 147–156.
2. L.J. Hanka, D.G. Martin, and G.L. Neil, *J. Nat. Prod.*, **41**, 85 (1978).
3. R.K. Elespuru and R.J. White, *Cancer Res.*, **43**, 2819 (1983).
4. R.K. Johnson, H.F. Bartus, G.A. Hofmann, J.O. Bartus, S.-M. Mong, L.F. Faucette, F.L. McCabe, J.A. Chan, and C.K. Mirabelli, in: "In Vitro and In Vivo Models for Detection of New Antitumor Drugs." Ed. by L.J. Hanka, T. Kondo, and R.J. White, Organising Committee of the 14th International Congress of Chemotherapy, Kyoto, 1986, pp. 15–26.
5. A.A.L. Gunatilaka, G. Samaranayake, D.G.I. Kingston, G. Hofmann, and R.K. Johnson, *J. Nat. Prod.*, **55**, 1648 (1992).
6. G.G. Harrigan, A. Ahmad, N. Baj, T.E. Glass, A.A.L. Gunatilaka, and D.G.I. Kingston, *J. Nat. Prod.*, **56**, 921 (1993).

7. C.E. Heltzel, A.A.L. Gunatilaka, T.E. Glass, D.G.I. Kingston, G. Hofmann, and R.K. Johnson, *J. Nat. Prod.*, **56**, 1500 (1993).
8. D.E.U. Ekong and A.U. Ogan, *J. Chem. Soc. C*, 311 (1968).
9. D.E.U. Ekong, E.O. Olagbemi, and F.A. Odutola, *Phytochemistry*, **8**, 1053 (1969).
10. C.M. Hasan, T.M. Healey, and P.G. Waterman, *Phytochemistry*, **21**, 1365 (1982).
11. D.F. Faulkner, V. Lebby, and P.G. Waterman, *Planta Med.*, **53**, 354 (1985).
12. J. Sinclair, *Gardens Bulletin Singapore*, **14**, 149 (1955); cited in A.K. Goel and S. Sharma, *Nord. J. Bot.*, **10**, 629 (1991).
13. J.C. Game, in: "Yeast Genetics; Fundamentals and Applied Aspects." Ed. by J.F.T. Spencer, D.M. Spencer, and A.R.W. Smith, Springer-Verlag, New York, 1983, pp. 109–137.
14. W.K. Eng, L. Faucette, R.K. Johnson, and R. Sternglanz, *Mol. Pharmacol.*, **34**, 755 (1988).
15. O. Castro, J. Lopez, and F.R. Stermitz, *J. Nat. Prod.*, **49**, 1036 (1986).
16. H. Guinaudeau, M. Leboeuf, and A. Cave, *J. Nat. Prod.*, **38**, 275 (1975).
17. S. Abd-El Atti, H.A. Ammar, C.H. Phoebe, P.L. Schiff, and D.J. Slatkin, *J. Nat. Prod.*, **45**, 476 (1982).
18. C.C. Hsu, R.H. Dobberstein, G.A. Cordell, and N.R. Farnsworth, *J. Nat. Prod.*, **40**, 152 (1977).
19. C.H. Phoebe, P.L. Schiff, J.E. Knapp, and D.J. Slatkin, *Heterocycles*, **14**, 1977 (1980).
20. G.A. Hofmann, C.K. Mirabelli, and F.H. Drake, *Anti-cancer Drug Design*, **5**, 273 (1990).
21. I. Borup-Grochtmann and D.G.I. Kingston, *J. Nat. Prod.*, **45**, 102 (1982).
22. P.E. Sonnet and M. Jacobson, *J. Pharm. Sci.*, **60**, 1254 (1971).
23. J. Cardellina, *J. Nat. Prod.*, **46**, 196 (1983).

Received 20 May 1993