Journal of Natural Products
Vol. 58, No. 9, pp. 1444-1449, September 1995

BIOACTIVE PRENYLHYDROQUINONE SULFATES AND A NOVEL C_{31} FURANOTERPENE ALCOHOL SULFATE FROM THE MARINE SPONGE, *IRCINIA* SP.

GIUSEPPE BIFULCO, INES BRUNO, LUIGI MINALE,* RAFFAELE RICCIO,

Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli Federico II, Via Domenico Montesano 49, 80131 Napoli, Italy

CÉCILE DEBITUS, GENIEVIEVE BOURDY,

Centre ORSTOM, B.P. A5, Nouméa, New Caledonia

AUDE VASSAS,

Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Université de Montpellier I, 15 av. Charles Flahault, 34060 Montpellier Cedex, France

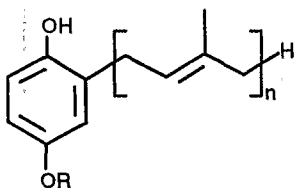
and JACQUES LAVAYRE

Rhône-Poulenc Rorer S.A., Centre de Recherche de Vitry-Alfortville, 94403 Vitry sur Seine Cedex, France

ABSTRACT.—Prenylhydroquinone sulfates have been isolated from a marine sponge, *Ircinia* sp., collected off New Caledonia. These compounds bind to the neuropeptide Y receptor, inhibit the tyrosine protein kinase and HIV-integrase enzymes, and co-occur with a mixture of sulfated prenylated chromanols and chromenols, prenylhydroquinones, and the corresponding quinones and chromenols. A hydroxylated heptaprenylhydroquinone, which proved to be active against tyrosine protein kinase, was also isolated, as well as a novel C_{31} furanoterpene alcohol sulfate, named ircinol sulfate.

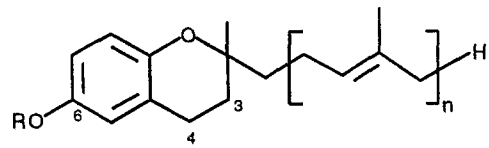
Sponges of the genus *Ircinia* are sources of linear polyprenyl benzoquinones and their corresponding quinols, with the latter usually present in much larger amounts (1,2). Polyprenylhydroquinones have also been reported from the related *Hippospongia communis* (3) and from a *Spongia* sp. (4). More recently, hexaprenylhydroquinone 1-sulfate has been isolated from a sponge of the genus *Dysidea* (5), while several prenylhydroquinone 4-sulfates, named sarcohydroquinone sulfates A-C, and the related chromenols, sarcochromenol sulfates A-C, have been isolated from the sponge *Sarcotragus spinulosus* (6). The prenylhydroquinone sulfates and the related chromenols were found to be inhibitors of H^+,K^+-ATPase (5,6).

In the course of our search for bioactive substances from New Caledonian marine invertebrates, we found that the $CHCl_3$ extract of a sponge of the genus *Ircinia*, which was collected in the Norfolk Ridge region at a depth of 425–500 meters, specifically inhibited the neuropeptide Y (NPY) receptor in vitro and also showed cytotoxicity against KB cells. From the sponge we have isolated the active principles, which were identified as penta-, hexa-, and heptaprenylhydroquinone 4-sulfates (**1–3**). Inhibition was observed with these compounds on the NPY receptor, and with the tyrosine protein kinase (TPK) and HIV-integrase enzymes. Further related metabolites isolated from the sponge were a mixture of prenylated benzopyran sulfates [**4** and **5**], the prenylated hydroquinones **7–9**, the corresponding quinones **10** and **11** (2,7), and the chromenols **12–14**, along with the hydroxylated 2-heptaprenylhydroquinone **6**, which also showed activity in the TPK and HIV-integrase inhibition assays. While these sponge metabolites are in part known and in part closely related to known marine natural products (1–7), compound **6** is new, and the pentaprenylhydroquinone sulfate **1** is a prenylated analogue of the known **2** and **3**. The quinols **8** and **9** are reported for the first time as pure compounds, having



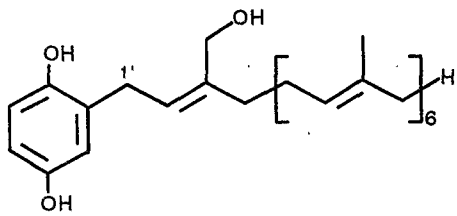
R=$SO_3^-Na^+$ R=H

1 n=5 **7** n=4
2 n=6 **8** n=5
3 n=7 **9** n=6

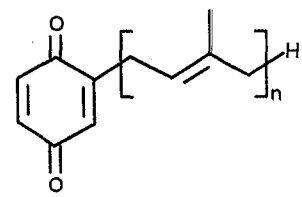


R=$SO_3^-Na^+$ R=H

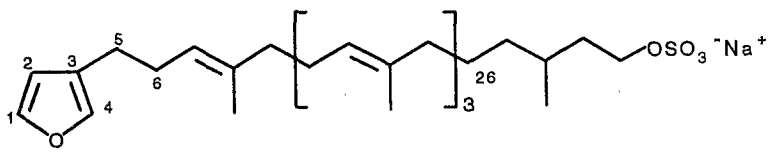
4 n=5 **12** n=4, $\Delta^{3,4}$
5 n=5, $\Delta^{3,4}$ **13** n=5, $\Delta^{3,4}$
14 n=6, $\Delta^{3,4}$



6



10 n=4
11 n=5



15

been obtained previously as mixtures of homologues (1), and the chromenols **12–14** are novel prenylated derivatives of dictyochromenol, an isoprenoid chromenol isolated from the brown alga *Dictyopteris undulata* (8). The sponge also yielded a novel C_{31} furanoterpenic alcohol sulfate, named ircinol sulfate [**15**], in small amounts.

The fabms (negative-ion mode) of the sulfated prenylhydroquinones **1–3** gave molecular anion peaks at *m/z* 529, 597, and 665 [MSO_3^-], respectively, consistent with penta-, hexa-, and heptaprenylhydroquinone sulfate structures. Their uv spectra (λ max 281 nm, ε 2700) were reminiscent of a hydroquinone chromophore (9), while the ir absorption at 1240 cm^{-1} indicated the presence of a sulfate moiety. The ^{1}H-nmr spectrum implied the presence of a monosubstituted hydroquinone [δ 7.03 (1H, d, *J*=2.7 Hz), 6.98 (1H, dd, *J*=2.7 and 8.6 Hz) and 6.71 (1H, d, *J*=8.6 Hz)] and of an all-trans prenyl moiety [δ 5.36 (1H, t, *J*=7.3 Hz, olefinic, H of the first isoprene unit, 3.30 (2H, d, *J*=7.3 Hz, ArCH_2CH=), 2.10 m (=C(Me)-CH_2CH_2-), 2.00 (t, *J*=7.5 Hz, =C(Me)-CH_2CH_2-), 1.73 (3H, s, methyl of the first isoprene unit), 1.69 and 1.63 (3H each, s, methyls of the terminal isoprene unit), 1.62 s (methyls in chain)]. The presence of the sulfate was confirmed by solvolysis in dioxane/pyridine (10) of the major compound **2** (2 mg in 0.2 ml of pyridine-dioxane, 1:1, heated at 130° for 2 h) affording the hexaprenylhydroquinone, eims *m/z* 518. The location of the sulfate at C-4 was derived from the ^{1}H-nmr downfield shifts observed for H-3 and H-5 in **2** in comparison with the desulfated sample (i.e., H-3: δ 7.03 in **2** vs. 6.57; H-5: δ 6.98 vs. 6.48, and H-6:

δ 6.71 vs. 6.61). Support came from comparison of the ^{1}H-nmr shifts of the aromatic protons in **2** with those of the hexaprenylhydroquinone 4-sulfate (6) and the isomeric 1-sulfate (5). The ^{13}C-nmr spectral data confirmed the structures proposed for compounds **1–3** and HETCOR experiments allowed the assignment of all carbons as reported in the Experimental. Compound **1** displayed affinity to the neuropeptide Y receptor with an IC_{50} value of 50.8 μg/ml and inhibited TPK with an IC_{50} value of 8 μg/ml and HIV-1 integrase (65% inhibition at 1 μg/ml). Compounds **2** and **3** showed similar activities, with compound **2** being more active, having an IC_{50} value of 4.0 μg/ml in the TPK enzymatic assay. Compound **3** exhibited an IC_{50} value of 8.0 μg/ml in this same bioassay.

A mixture of chromanol **4** and chromenol **5**, obtained as a single peak by reversed-phase hplc, proved to be resistant to separation attempts. Because of the limited amount (2.3 mg) we pursued characterization on the mixture. The fabms gave two molecular anion peaks at *m/z* 597 and 595 [MSO_3^-]. The ^{1}H-nmr spectrum showed three doubled aromatic signals centered at δ 6.69 (each d, J=2.8 Hz, H-5), 7.00 (each dd, J=8.5 and 2.8 Hz, H-7), and 7.04 (each d, J=8.5 Hz, H-8). In addition, the ^{1}H-nmr spectrum contained two olefinic proton doublets at δ 6.40 and 5.69 (J=9.9 Hz) coupled to one another, which could be assigned to the $\Delta^{3,4}$- protons of a chromenol, supported by a singlet at δ 1.38 assigned to the methyl attached to the oxygen-bearing carbon of the pyran ring. In the high-field region of the ^{1}H-nmr spectrum, a triplet at δ 2.80 (J=6.5 Hz, benzylic methylene) along with a methyl singlet at δ 1.29, were suggestive of the presence of the corresponding chromanol (11). The remaining signals, at δ 5.14 m, 2.10 m, 2.01 m, 1.70 s, 1.63 s, and 1.62 s could be assigned to an all-trans polyprenyl side-chain, identified as a hexaprenyl chain by ms. These data indicated that this material was a mixture of **4** and **5**. The presence of the sulfate group was confirmed by solvolysis in dioxane/pyridine (see below) affording a mixture of a chroman-6-ol and a chromen-6-ol (fabms *m/z* 517 and 515), whose aromatic proton signals were observed shifted upfield to δ 6.48, 6.55, and 6.65. The chromenol sulfate **5** is a known compound (6).

The ^{1}H-nmr spectrum (CD_3OD) of compound **6** implied that it had a prenylated hydroquinone structure, with significant signals occurring at δ 6.61 (d, J=8.5 Hz, H-6), 6.57 (d, J=3.0 Hz, H-3), 6.48 (dd, J=8.5 and 3.0 Hz, H-5), 5.45 t (J=7.8 Hz) and 5.13 m (olefinics), 2.10 and 2.01 m (vinyl methylenes), 1.70 s (cis methyl of the terminal isoprene unit), and 1.62 s (olefinic methyls). A 2H singlet at δ 4.24 was indicative of the presence of an allylic primary hydroxyl group, supported by a methylene carbon signal at δ 60.3 ppm. The fabms exhibited a quasimolecular ion at *m/z* 533 $[M-H]^-$, corresponding to a heptaprenylhydroquinone in which one of the methyls has been oxidized to hydroxymethylene. The absence of a signal for the methyl group located on the first isoprene unit at δ 1.73 along with the shift of the olefinic proton of the first isoprene unit to δ 5.45 (vs. 5.36 in prenylhydroquinones) led to the location of the hydroxyl group as in **6**. An intense nOe between the hydroxymethylene protons (δ 4.24) and the C-1′ methylene proton doublet (δ 3.36) confirmed the location of the hydroxyl group and also demonstrated the trans- stereochemistry of the double bond. The *E* geometry of the remaining five unsymmetrically substituted double bonds was derived from the shielded ^{13}C-nmr chemical shifts of the olefinic methyl signal appearing at δ 16.1 ppm. Compound **6** was inhibitory in the TPK assay with an IC_{50} value of 5.9 μg/ml and was also active in the HIV-1 integrase assay at 5 μg/ml (45% inhibition). An octaprenylhydroquinone with the methyl group of the fifth isoprene

unit oxidized to hydroxymethylene has been isolated from the Mediterranean sponge *Ircinia spinosula* (1).

The eims of the prenyl chromenols **12–14** gave molecular ion peaks at *m/z* 448, 516, and 584. Inspection of the ^{1}H-nmr data in each case revealed resonances indicative of a 1,2,4-trisubstituted aromatic ring [δ 6.67 (d, J=8.5 Hz), 6.58 (dd, J=8.5 and 3.0 Hz), 6.48 (d, J=3.0 Hz)]; a conjugated disubstituted double bond [δ 6.28 (d, J=9.9 Hz), 5.62 (d, J=9.9 Hz)]; and a methyl on a quaternary carbon [δ 1.38 s]. These data, with support from the ^{13}C-nmr spectra (see Experimental), implied a chromenol structure (8). The remaining signals of the nmr spectra of **12–14** were assigned to the polyprenyl chains [δ 5.15 (q, J=6.7 Hz, olefinic H); 2.05 and 2.00 (each m, CH_2); 1.69 s (cis methyl of the terminal isoprene units), 1.62 (methyls in side-chain) and 1.63 s (trans methyl of the terminal isoprene units)]. The side-chain lengths were inferred from the mass spectra. While a sesquiterpene chromenol has been reported from a brown alga (8), to our knowledge this is the first report of the occurrence of penta-, hexa-, and heptaprenylated homologues. The chromenol derivatives **12–14** were submitted to various receptor-binding assays and enzymatic assays (see Experimental) but proved to be inactive.

The fabms (negative-ion mode) of compound **15**, a novel furanoterpene alcohol sulfate we have called ircinol sulfate, exhibited a molecular anion peak at *m/z* 533 [MSO_3^-]. Key features in the ^{1}H-nmr spectrum of **15** were attributed to a β-substituted furan moiety [δ 7.39 (br s, H-1), 7.26 (br s, H-4), 6.32 (br s, H-2)]; four trisubstituted *E* double bonds [δ 5.20 (1H, t, J=7 Hz, H-7), 5.14 (3H, m)] with associated olefinic methyls [δ 1.62 s]; an oxymethylene group [δ 4.06 m]; and a secondary methyl [δ 0.95 d]. Signals at δ 2.47 (2H, t, J=7.5 Hz, H-5), 2.27 (2H, q, J=7.5 Hz, H-6), 2.11 m, and 2.01 m were assigned to the allylic methylene protons. Assignment of the unconjugated C-25–C-31 terminus was established by ^{1}H-^{1}H COSY spectroscopy; H_2-25: 2.01, H_2-26: 1.44–1.33, H_2-27: 1.35–1.16, H-28: 1.65, H_3-29: 0.95, H_2-30: 1.72–1.46, and H_2-31: 4.06 ppm. The presence of a sulfate group was indicated by the ir absorption at 1240 cm^{-1} and confirmed by desulfation in pyridine/dioxane at 120° affording the desulfated alcohol (eims *m/z* 454, ^{1}H nmr, δ CH_2OH, 3.70). A linear C_{31} difuranoterpene, difurospinosulin, previously isolated from *Ircinia spinosula*, was suggested to be derived presumably from a C_{35} linear furanoterpene by the loss of four carbon atoms (1). Ircinol sulfate [**15**] is the second example of a C_{31} furanoterpene encountered in marine sponges.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AMX-500 instrument [^{1}H at 500 MHz, ^{13}C at 125 MHz, δ (ppm), J in Hz]. Spectra in CD_3OD are referenced to the CHD_2OD signal at 3.34 ppm and to the central CD_3OD carbon signal at 49.0 ppm, while spectra in $CDCl_3$ are referred to the $CHCl_3$ signal at 7.27 ppm and to the central $CDCl_3$ carbon signal at 77.0 ppm. Fabms were measured in the negative-ion mode on a VG ZAB instrument equipped with a fab source [in glycerol or glycerol-thioglycerol (3:1) matrix, Xe atoms of 2–6 kV]; eims were measured on a Kratos MS-50 instrument at 70 eV. Uv spectra were taken on a Beckman DU70 spectrometer and Ft-ir spectra were recorded on a Bruker IFS-48 spectrometer as KBr pellets.

ANIMAL MATERIAL.—Specimens of *Ircinia* sp. were collected by dredging off New Caledonia in the Norfolk Ridge region (23°37,8′ S 167°37,8′ S) at a depth of 425–500 m. This specimen measures 120 mm long, 90 mm wide, and 50 mm in height; it consists of many irregular anastomosed branches, 5 to 10 mm in diameter. Spaces between branches: 5–20 mm wide. The free part of the branches is 5–30 mm in length, with a conical apex. Conules are low, 2–3 mm apart. Oscules are inconspicuous and the texture is compressible, resistant, and leathery. The surface is smooth, but the conules are connected by thin ridges. The ectosomal membrane is very tough, including many filaments. [Main fibers, generally uncored: 80–120 μm in diameter, slightly fasciculate; sec-

ondary fibers very short: 20 μm in diameter. The specimen is full of filaments: 2.5 mm thick in the middle region]. A sample is preserved at the Centre ORSTOM, Nouméa, New Caledonia, under reference number R1518.

EXTRACTION AND ISOLATION.—The lyophilized sponge (350 g) was sequentially extracted with petroleum ether (5.8 g of extract), CH_2Cl_2 (2.15 g extract), CH_2Cl_2-MeOH 4:1 (13.6 g of extract), and MeOH. The petroleum ether-soluble material (5.8 g) was purified by mplc on Si gel with a stepwise gradient of hexane and EtOAc, followed by hplc on a Partisil 10 column with hexane-EtOAc (49:1) to give the prenylated quinones **10** (45 mg) and **11** (55 mg), and the related chromenols **12** (75 mg), **13** (94 mg), and **14** (21 mg).

Compound **10**.—Eims (70 eV) *m/z* 516 $[M]^+$; uv (MeOH) λ max (log ε) 214 (4.00), 294 (3.57), 316 (3.36) nm; ir (KBr pellet) ν max 1660, 1600 cm^{-1}; ^1H nmr δ 6.76 (d, J=10.1 Hz), 6.71 (dd, J=10.1 and 2.4 Hz), 6.54 (d, J=2.4 Hz), 5.16 (t, J=7.3 Hz), 5.12 (m), 3.14 (d, J=7.3 Hz), 2.08 (m), 2.00 (m), 1.69 (s), 1.64 (s), 1.60 (s).

Compound **11**.—Eims (70 eV) *m/z* 584 $[M]^+$.

Compound **12**.—Eims (70 eV) *m/z* 448 $[M]^+$; uv (MeOH) λ max (log ε) 235 (4.20), 262 (3.65), 331 (3.63) nm.

Compound **13**.—Eims (70 eV) *m/z* 516 $[M]^+$.

Compound **14**.—Eims (70 eV) *m/z* 584 $[M]^+$ ^{13}C nmr ($CDCl_3$, 125.76 MHz) δ 149.2 (C-6), 146.9 (C-8a), 131.3 (C-4a), 130.9 (C-4), 124.3 and 135.3 (olefinic carbons), 122.5 (C-5), 116.7 (C-8), 115.3 (C-7), 112.8 (C-3), 78.1 (C-2), 40.1 and 26.7 (methylenes in side-chain), 26.0 (2-Me), 25.7 (cis methyl of the terminal isoprene units), 17.7 (trans methyl of the terminal isoprene units), 16.1 (methyls in side-chain).

The CH_2Cl_2/MeOH-soluble material (13.9 g) was subjected to mplc on Si gel with increasing amounts of MeOH in $CHCl_3$. The fraction eluted with $CHCl_3$ yielded the prenylated hydroquinones **7** (0.55 mg), **8** (1.12 mg), and **9** (1.29 mg); the fractions eluted with $CHCl_3$/MeOH were then separated by reversed-phase hplc on a C_{18} μ-Bondapak column with MeOH and increasing amounts of H_2O to give the novel C_{31} furanoterpene alcohol sulfate, ircinol sulfate [**15**] [eluted with MeOH-H_2O, 9:1) (2.6 mg)], the hydroxylated-2-heptaprenylhydroquinone **6** (3.4 mg), and a mixture of the sulfated chromanol and chromenol **4** and **5** (2.3 mg), eluted with MeOH-H_2O (85:15), and finally the active prenylhydroquinone sulfates **1** (4 mg), **2** (13 mg), and **3** (6 mg), eluted with MeOH-H_2O (4:1).

Compound **7**–**9**.—Eims (70 eV) *m/z* 518, 586, and 654, respectively; ^1H nmr δ 6.68 (d, J=8.5 Hz), 6.54 (d, J=3.0 Hz), 6.48 (dd, J=8.5 and 3.0 Hz), 5.40 (t, J=7.5 Hz), 5.12 (m), 3.30 (d, J=7.5 Hz), 2.10 (m), 2.01 (m), 1.70 (s), 1.64 (s), 1.60 (s).

Compound **15**.—^{13}C nmr ($CDCl_3$, 125.76 MHz) δ 140.1 (C-1), 112.0 (C-2), 125.2 (C-3), 143.7 (C-4), 37.5 (C-5), 26.0 (C-6), 125.5 (C-7), 135.9 (C-8), 16.1 (olefinic methyls, *E* geometry), 37.7 (C-25), 26.4 (C-26), 29.6 (C-27), 30.7 (C-28), 19.9 (C-29), 37.5 (C-30), 67.5 (C-31).

Compound **2**.—^{13}C nmr ($CDCl_3$, 125.76 MHz) δ 153.5 (C-1), 129.8 (C-2), 123.9 (C-3), 146.4 (C-4), 120.8 (C-5), 115.6 (C-6), 29.2 (C-1′), 123.5 (C-2′), 132.0 (C-3′), 40.8, 27.5 (methylenes in side-chain), 25.9 (cis methyl of the terminal isoprene unit), 17.5 (trans methyl of the terminal isoprene unit), 16.3 (methyl on the first isoprene unit), 16.1 (methyls in side-chain).

BIOLOGICAL TESTING.—The in vitro binding assays were performed with membrane preparations from animal tissues (rats or guinea pigs) or cell lines (cells expressing a human gene) according to standard methods (12). The membrane preparations were incubated under optimal pH, temperature, time and media conditions, in the presence of the specific radiolabeled ligand with a given concentration of the test compounds or with a compound possessing a high affinity for the corresponding binding site (non-specific binding measurement). After equilibrium, the mixtures were filtered through glass fiber filters and the radioactivity remaining in the filter was measured with a scintillator counter.

The following transmitters were used: somatostatin (13), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY) (14), bradykinin (human B2 receptor) (15), neurotensin (NT), galanin, and the senektide (NK3) binding assays. In particular, the NPY binding assay was performed with membranes from rat cerebral cortices as sources of receptors and [^{3}H]-NPY as radiolabeled ligands. The bradykinin binding assay was performed with the membranes from SF21 cells infected by baculovirus expressing B2 bradykinin receptors and [^{3}H]-bradykinin. The inhibition of TPK was measured using an ELISA methodology. Our assay contained a 3T3 overexpressing HER2 membrane fraction as enzyme and poly (Glu6, Ala3, Tyr 1) as a substrate. The inhibition of HIV-1 integrase was measured using scintillation proximity assay (SPA) methodology.

ACKNOWLEDGMENTS

We thank Professor C. Lévi, Muséum Nationel d'Histoire Naturelle, Paris, France, for the identification of the sponge. This contribution is part of the ORSTOM-CNRS project "Substance Marines d' Intérêt Biologique" (SMIB). The chemical work was supported by MURST (Ministero per l'Università e la Ricerca scientifica) and C.N.R.

(contributi no. 92.00531 e 93.03093), Rome, Italy. Mass spectra were provided by "Servizio di Spettrometria di Massa del C.N.R. e dell'Università di Napoli," whose assistance is gratefully acknowledged. Nmr spectra were performed at "Centro di ricerca interdipartimentale de analisi strumentale" (CRIAS) of the Faculty of Pharmacy, University of Naples. The receptor binding and enzymatic inhibition assays were done by the biological screening department of Rhône-Poulenc Rorer, Centre de Recerche de Vitry-Alfortville, France.

LITERATURE CITED

1. G. Cimino, S. De Stefano, and L. Minale, *Tetrahedron,* **28**, 1315 (1972).
2. G. Cimino, S. De Stefano, and L. Minale, *Experientia,* **28**, 1401 (1972).
3. Y.F. Pouchus, J.F. Verbist, J.F. Biard, and K. Boukef, *J. Nat. Prod.,* **51**, 188 (1988).
4. D. Lumsdon, R.J. Capon, S.G. Thomas, and A.A. Beveridge, *Aust. J. Chem.,* **45**, 1321 (1992).
5. N. Fusetani, M. Sugano, S. Matsunaga, K. Hashimoto, H. Shikama, A. Ohta, and H. Nagano, *Experientia,* **43**, 1233 (1987).
6. V.A. Stonik, T.N. Makarieva, and D. Dmitrenok, *J. Nat. Prod.,* **55**, 1256 (1992).
7. G. Cimino, P. De Luca, S. De Stefano, and L. Minale, *Tetrahedron,* **31**, 271 (1975).
8. M.N. Dare, T. Kusmi, M. Ishitsuka, T. Iwashita, and H. Kakisawa, *Heterocycles,* **22**, 2301 (1984).
9. A.I. Scott, "Interpretation of the Ultra-Violet Spectra of Natural Products," Pergamon Press, London, 1964, p. 91.
10. J. McKenna and J.K. Norymberski, *J. Chem. Soc. C,* 3889 (1972).
11. G. Cimino, S. De Rosa, S. De Stefano, R. Morrone, and G. Sodano, *Tetrahedron,* **41**, 1093 (1985).
12. E.C. Hulme, "Receptor-Ligand Interactions: A Practical Approach." IRL Press, Oxford University Press, Oxford, UK, 1992.
13. V.T. Tran, M.F. Beal, and J.B. Martin, *Science,* **228**, 492 (1985).
14. A. Unden, K. Tatemoto, V. Mutt, and T. Barttfai, *Eur. J. Biochem.,* **145**, 525 (1984).
15. R.B. Innis, D.C. Manning, J.M. Stewart, and S.H. Snyder, *Proc. Natl. Acad. Sci.,* **78**, 2630 (1981).

Received 22 December 1994