Journal of Natural Products
Vol. 51, No. 6, pp. 1098-1103, Nov-Dec 1988

POLAR STEROIDS FROM THE MARINE SCALLOP *PATINOPECTEN YESSOENSIS*

MARIA IORIZZI, LUIGI MINALE,* RAFFAELE RICCIO,

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, via D. Montesano, 49, 80131 Napoli, Italy

JONG-SOO LEE, and TAKESHI YASUMOTO

Faculty of Agriculture, Tohoku University, Tsutsumidori, Amamiyamachi, Sendai 980, Japan

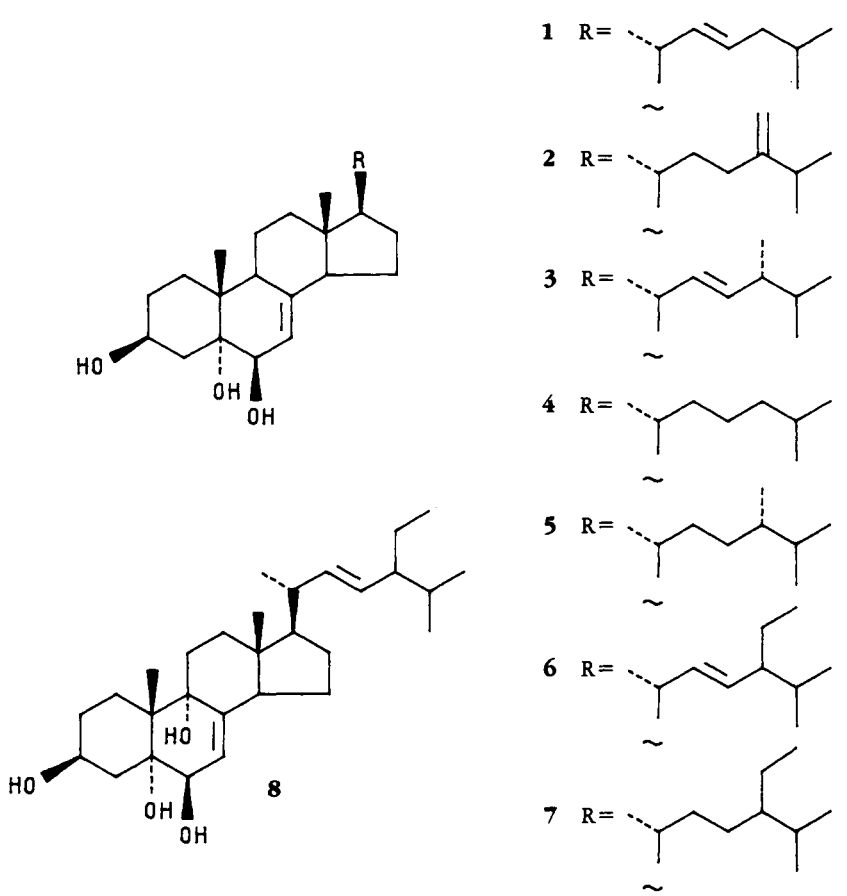
ABSTRACT.—Eight polyhydroxylated sterols, seven of them novel, have been isolated from the hepatopancreas of the scallop *Patinopecten yessoensis*. Compounds **1–7** possessed the same Δ^7-3β,5α,6β-triol nucleus but differed in the side chains. One very minor component **8** had an additional hydroxyl group at C-9α. Their structures were deduced from spectral data and by comparison with synthetic model compounds.

In connection with a major interest in marine toxins and particularly in "diarrethic shellfish toxins" (1,2), the hepatopancreas (50 kg) of scallops (*Patinopecten yessoensis* Jay; class Bivalvia) was extracted with Me_2CO at room temperature. While purifying the toxins, we also obtained a polar steroid fraction. In this paper we report on seven novel polyhydroxysteroids **1**, **2**, **4–8** along with the known **3** isolated from the digestive glands of the scallop *P. yessoensis*.

Purification of the steriods in the Et_2O-soluble residue of the extract was achieved by chromatography on Si gel in C_6H_6-hexane (9:1), followed by gel permeation through Sephadex LH-20 in C_6H_6-MeOH (1:1) and then chromatography on Si gel in $CHCl_3$-MeOH-H_2O (85:15:1) to give a major polar sterol fraction (60 mg). The crude sterol mixture was separated into individual compounds by repeated reversed-phase hplc using MeCN/H_2O and MeOH/H_2O systems. Because the spectral data indicated that the major components **1–7** of these polar fractions possessed virtually identical nuclei but different side chains, it was only necessary to establish the nuclear substitution pattern for the most abundant sterol **2**.

STRUCTURE ELUCIDATION OF 24-METHYLENECHOLEST-7-ENE-3β,5α,6β-TRIOL [**2**].—The mass spectrum displayed an ion peak at *m/z* 412 corresponding to the loss of a molecule of H_2O from the molecule. Successive losses of 18 mass units (*m/z* 394 and *m/z* 376) indicated the presence of three hydroxyl groups. DEPT ^{13}C-nmr spectrum in CD_3OD confirmed the presence of three hydroxyl groups, two secondary and one tertiary, with signals at 68.4 (CH), 74.4 (CH), and 77.0 (C) ppm. The 250-MHz ^{1}H-nmr spectrum of **2** in CD_3OD indicated the presence of a terminal methylene group (2H, 4.68 and 4.76 ppm) and showed the presence of an additional trisubstituted double bond (1H, five lines signal with separations of 2 Hz at 5.32 ppm), as well as a terminal isopropyl group (3H, d, $J = 6.8$ at 1.06 ppm; 3H, d, $J = 6.8$ at 1.07 ppm; and 1H, quintet, $J = 6.8$ at 2.27 ppm). Also present were two 3H singlets at 0.67 ppm (18-Me) and 1.09 (19-Me) ppm and one 3H doublet at 1.02 (21-Me) ppm. Two one-proton signals at 3.57 and 4.01 ppm were assigned to the hydroxymethine protons.

In a double resonance experiment, irradiation at 5.32 ppm transformed the broad doublet ($J = 4$ Hz) at 3.58 ppm into a broad singlet, thus indicating that the olefinic proton is located next to a hydroxyl, the latter being adjacent to a quaternary carbon. The multiplet centered around 4.01 ppm had a complexity (3) normally seen for a 3α-carbinol proton, and the unusually high chemical shift suggested the additional tertiary alcohol was located at C-5. These data were suggestive of a Δ^7-3, 5,6-triol structure or, alternatively, a $\Delta^{9(11)}$-3,5,12-triol structure. Examination of the nmr signals of the C-



18 and C-19 protons, even if their chemical shifts were in better agreement with those expected for a Δ^7-3β,5α,6β-triol structure (4,5), did not permit rigorous exclusion of an alternative structure. Thus, it was decided to synthesize the known 5α-ergosta-7,22-diene-3β,5,6α (and β)-triols (6–8). Treatment of ergosterol with 1 mol of *m*-chloroperbenzoic acid to obtain the Δ^7-3β,5α,6α-triol-6-*m*-chlorobenzoate followed by hydrolysis (KOH/MeOH) to the corresponding triol (6,7) and chromatographic purification on Si gel also gave in very small amounts the isomeric Δ^7-3β,5α,6β-triol.

^{1}H- and ^{13}C-nmr comparison of the natural steroid with the synthetic triols definitively established the Δ^7-3β,5α,6β-triol structure for **2**. Major differences in the ^{1}H-nmr spectra of the synthetic compounds were the chemical shift of C-19, C-6, and C-7 protons (1.02, 3.93, and 5.00 ppm in the spectrum of the 6α-OH isomer and 1.09, 3.57, and 5.30 ppm in the spectrum of the 6β-OH isomer). The 24-methylene side chain structure in **2** received confirmation from the ^{13}C-nmr data (see Experimental); assignments of carbons C-20 to C-28 were based on analogy to the known values for 24-methylene steroid derivatives (9–11).

Table 1 shows the ^{1}H-nmr chemical shifts and observed multiplicity of sterols **1–8**. These data show conclusively that they all have the Δ^7-3β,5α,6β-triol structure but different side chains. The identification of the side chains was achieved mainly on the basis of ^{1}H nmr (12). The spectral data for **3** were coincident with those of synthetic 5α-ergosta-7,22-diene-3β,5,6β-triol; the assignment of 24*S* configuration to **5** was based on analogy with **3**. The ^{1}H-nmr spectrum of the more polar compound **8** was similar to that of **6**, with differences for Me-18 and Me-19 (i.e., 0.70 and 1.15 ppm in **8** vs. 0.67

TABLE 1. Selected ^{1}H-nmr Chemical Shifts (250 MHz, CD_3OD) of Δ^7-3β,5α,6β-Triols **1**–**7** and Δ^7-3β,5α,6β,9α-Tetraol **8**.[a]

Proton	Compound							
	1	**2**	**3** natural and synthetic	**4**	**5**	**6**	**7**	**8**
H-3	4.01 m	4.01 m	4.01 m	4.01 m	4.01 m	4.01 m	4.01 m	4.01 m
H-6	3.57 bd (4)	3.57 bd (4)	3.57 bd (4)	3.57 bd (4)	3.57 bd (4)	3.57 bd (14)	3.57 bd (4)	3.68 dd (2,4)
H-7	5.30 m (W½ 10)	5.30 m (W½ 10)	5.30 m (W½ 10)	5.30 m (W½ 10)	5.30 m (W½ 10)	5.30 m (W½ 10)	5.30 m (W½ 10)	5.36 dd (2,4)
H-18	0.68 s	0.67 s	0.68 s	0.67 s	0.67 s	0.68 s	0.67 s	0.70 s
H-19	1.09 s	1.09 s	1.09 s	1.09 s	1.09 s	1.09 s	1.09 s	1.15 s
H-21	1.07 d (7)	1.02 d (6.8)	1.07 d (7)	0.99 d (6.5)	0.98 d (6.5)	1.07 d (6.5)	0.98 d (7)	1.08 d (6.5)
H-26,27	0.91 6H d (6.5)	1.06 d (6.8)	0.86 d (7)	0.92 6H d (6.5)	0.84 d (6.5)	0.86 d (6.5)	0.84 d (6.5)	0.87 d (7)
		1.07 d (6.8)	0.90 d (6.5)		0.86 d (7)	0.88 d (7)	0.86 d (7)	0.89 d (6.5)
H-28	—	4.68 brs	0.96 d (7)	—	0.90 d (7)	—	—	—
		4.76 brs						
Other signals	22,23-H: 5.20-5.35 m	—	22,23-H: 5.23 m	—	—	22,23-H: 5.13 dd (15,7.5) 5.20 dd (15,8) 29-H: 0.92 t (7)	29-H: 0.91 t (7)	22,23-H: 5.10 dd (15,7.5) 5.20 dd (15,8) 29-H: 0.92 t (7)

[a]The chemical shift values are given in ppm and were referred to CD_3OD (3.34 ppm). The coupling constants are given in Hertz and are enclosed in parentheses.

and 1.09 ppm in **6**) and H-6 and H-7 [i.e., 3.68 (dd, $J = 4$, 2 Hz) and 5.36 (dd, $J = 4$, 2 Hz) ppm in **8** vs. 3.58 and 5.30 ppm in **6**] signals. The H-6 and H-7 appeared as two sharp double doublets indicating the presence of only one proton in the allylic position relative to H-7 and in homoallylic position relative to H-6, respectively, and thus suggesting one *tert* hydroxyl group at C-9 or C-14. The presence of one extra hydroxyl group in **8** was in agreement with its polarity and mass spectral data. In the ei mass spectrum of **8**, an ion peak was observed at *m/z* 442, corresponding to a dehydroderivative of **6**, and assignable to $[M - H_2O]^+$. The introduction of the extra *tert*-hydroxyl group also affected significantly the chemical shift of C-19 methyl protons, which displayed a downfield shift of 0.06 ppm relative to **6**, in agreement with the location of a hydroxyl group at C-9 (4,13). Thus, the structure of **8** was deduced to be 24 (ξ)-ethylcholesta-7,22-diene-3β,5α,6β,9α-tetraol.

5α-Ergosta-7,22-diene-3β,5,6β-triol [**3**] (cerevisterol) has been described as a minor yeast and ergot sterol (14); it has been recently isolated from the wood-rotting fungus *Polyporus versicolor* (13), which also yielded in smaller amounts the 5α-ergosta-7,22-diene-3β,5,6β,9α-tetraol along with other Δ^7,9α-OH oxygenated derivatives of ergosterol.

The Δ^7-3β,5α,6β-trihydroxysteroids seem quite obviously to originate from $\Delta^{5,7}$-3β-hydroxysterols, and the fact that the presently described *Patinopecten* polyhydroxysterols all possess the same nucleus and different side chains seems to indicate that the organism possesses the enzyme system to convert dietary $\Delta^{5,7}$-3β-hydroxysterols into the same polyhydroxylated system. In this connection it is surprising to note that $\Delta^{5,7}$-3β-hydroxysterols have only occasionally been detected in scallops (15) and have never been described among sterols of *P. yessoensis* (16,17).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: nmr, Bruker WM-250; ms, Kratos MS 30 mass spectrometer; optical rotations, taken in MeOH, Perkin-Elmer model 141 polarimeter; hplc, Waters Model 6000 A pump equipped with a U6K injector and a Model 401 differential refractometer detector; C_{18} μ-Bondapak columns (30 cm × 7.8 mm i.d. and 30 cm × 3.9 mm i.d.).

EXTRACTION OF THE DIGESTIVE GLANDS OF *P. YESSOENSIS*.—The digestive glands (50 kg) of the scallop *P. yessoensis* collected in 1985 at Mutsu Bay, Japan (a reference specimen is deposited at the Faculty of Agriculture, Tohoku University), were extracted with Me_2CO at room temperature. After removal of the organic solvent, the aqueous suspension was extracted with Et_2O. The Et_2O-soluble material was chromatographed on a Si gel column (Wakogel C-100) eluted with C_6H_6-hexane (1:1), C_6H_6, and C_6H_6-MeOH (9:1). The combined C_6H_6-MeOH (9:1) eluate was chromatographed on a column of Sephadex LH-20 (2.8 × 120 cm) washed with C_6H_6-MeOH (1:1). The fractions eluted between 280 and 320 ml were combined and dissolved in MeOH and successively purified on a column of Si gel (Kiesel gel 60, Merck, 230–400 mesh, 0.5 × 100 cm) eluted with $CHCl_3$-MeOH-H_2O (85:15:1) to give three fractions (uv detector). Fraction 3 contained 60 mg of a mixture of complex polar steroids.

SEPARATION OF THE POLYHYDROXYLATED STEROL MIXTURE.—The polar fraction of the polyhydroxysterols was dissolved in MeCN and subjected to preparative reversed-phase hplc on a C_{18} μ-Bondapak column with 73% aqueous MeCN as the mobile phase to give five major peaks: peak 1 contained a mixture of **8**, **1**, and **2**; peak 2 contained a mixture of **3** and **4**; peaks 3, 4, and 5 contained single compounds, **5**, **6**, and **7**, respectively. Further purification of the sterol mixture was achieved by hplc on an analytical C_{18} μ-Bondapak column eluted with 83% aqueous MeOH to give pure **1**, **2**, **3**, **4**, and **8**. All sterols were obtained in milligram amounts, the most abundant being **2** (3.5 mg) and the least abundant being **8** (0.8 mg).

Cholesta-7,22E-diene-3β,5α,6β-triol [**1**].—$[\alpha]_D$ −25.0°; eims *m/z* (rel. int.) $[M - H_2O]^+$ 398 (40), 380 (80), 362 (100%), 347 (30); ^1H nmr see Table 1.

24-Methylenecholest-7-ene-3β,5α,6β-triol [**2**].—$[\alpha]_D$ −26°; eims *m/z* (rel. int.) $[M - H_2O]^+$ 412 (50), 394 (80), 379 (100%), 376 (70); ^{13}C nmr (62.9 MHz, CD_3OD) δ 31.8 (C-1), 34.0 (C-2), 68.4 (C-3), 40.9 (C-4), 77.0 (C-5), 74.4 (C-6), 119.2 (C-7), 143.8 (C-8), 44.5 (C-9), 38.2 (C-10), 23.0 (C-11), 40.6 (C-12), 44.9 (C-13), 55.9 (C-14), 24.0 (C-15), 28.7 (C-16), 57.6 (C-17), 12.4 (C-18), 18.8 (C-19),

37.2 (C-20), 19.3 (C-21), 36.0 (C-22), 32.1 (C-23), 158.0 (C-24), 35.0 (C-25), 23.3 (C-26), 22.4 (C-27), 106.9 (C-28); ^{1}H nmr see Table 1.

(24R)-24-Methylcholesta-7,22E-diene-3β,5α,6β-triol [**3**].—$[\alpha]_D$ −16.5°; eims (rel. int.) *m/z* $[M-H_2O]^+$ 412 (55), 394 (65), 379 (100%), 376 (70); ^{1}H nmr see Table 1.

Cholest-7-ene-3β,5α,6β-triol [**4**].—$[\alpha]_D$ −20.8° [lit. (6) −60° in pyridine]; eims *m/z* $[M-H_2O]^+$ 400 (30), 382 (75), 367 (100%), 364 (50).

(24S)-24-Methylcholest-7-ene-3β,5α,6β-triol [**5**].—$[\alpha]_D$ −10°; eims (rel. int.) *m/z* $[M-H_2O]^+$ 414 (65), 396 (90), 381 (100%), 378 (80).

24-Ethylcholesta-7,22E-diene-3β,5α,6β-triol [**6**].—$[\alpha]_D$ −4.6°; eims (rel. int.) *m/z* $[M-H_2O]^+$ 426 (60), 408 (90), 393 (100%), 390 (75).

24-Ethylcholest-7-ene-3β,5α,6β-triol [**7**].—$[\alpha]_D$ −12.7°; eims (rel. int.) *m/z* $[M-H_2O]^+$ 428 (50), 410 (90), 395 (100%), 392 (75).

24-Ethylcholest-7-ene-3β,5α,6β,9α-tetraol [**8**].—$[\alpha]_D$ −66.5°; eims (rel. int.) *m/z* $[M-H_2O]^+$ 442 (30), 424 (100%), 406 (90).

5α-Ergosta-7,22-diene-3β,5,6α-triol and 5α-Ergosta-7,22-diene-3β,5α,6β-triol.—We have followed the procedure of Windhaus and Luttringhaus (6) to obtain 5α-ergosta-7,22-diene-3β,5,6α-triol-6-*m*-chlorobenzoate (18) from ergosterol (2 g). The product, without further purification, was hydrolyzed by treatment with 10% methanolic KOH at reflux temperature for 2 h. Normal workup and Si gel cc in $CHCl_3$ and $CHCl_3$-MeOH (from 98:2 to 95:5) gave the major 5α-ergosta-7,22-diene-3β,5,6α-triol (0.87 g), mp 246°; eims *m/z* $[M-H_2O]^+$ 412 (60), 394 (70), 379 (100%); ^{1}H nmr (250 MHz, CD_3OD) δ 5.25 (2H, m, H-22,23), 5.00 (1H, broad signal, W½ = 5 Hz, H-7), 3.93 (2H, m, H-3α,6β), 1.07 (3H, d, *J* = 6 Hz, H_3-21), 1.02 (3H, s, H_3-19), 0.97 (3H, d, *J* = 7.0 Hz, H_3-28), 0.87 and 0.89 (each 3H, d, *J* = 6.5 Hz, H_3-26,27), 0.63 (3H, s, H_3-18); ^{13}C nmr (62.9 MHz, CD_3OD) δ 31.6 (C-1), 32.8 (C-2), 68.1 (C-3), 40.0 (C-4), 76.8 (C-5), 71.4 (C-6), 120.6 (C-7), 142.9 (C-8), 44.6 (C-9), 39.7 (C-10), 22.3 (C-11), 40.7 (C-12), 44.8 (C-13), 55.9 (C-14), 23.8 (C-15), 29.1 (C-16), 57.4 (C-17), 12.7 (C-18), 18.2 (C-19), 41.5 (C-20), 21.6 (C-21), 136.9 (C-22), 133.4 (C-23), 44.3 (C-24), 34.3 (C-25), 20.0 (C-26), 20.4 (C-27), 18.1 (C-28). Also obtained was a minor amount (9 mg after hplc purification on a C_{18} μ-Bondapak column, 75% aqueous MeOH) of the isomer 5α-ergosta-7,22-diene-3β,5,6β-triol, mp 253–255° [lit. (14) 252–255°]; eims *m/z* $[M-H_2O]^+$ 412 (55), 394 (65), 379 (100%); ^{1}H nmr (250 MHz) see Table 1; ^{13}C nmr (62.9 MHz, CD_3OD) δ 31.8 (C-1), 34.0 (C-2), 68.5 (C-3), 40.7 (C-4), 77.0 (C-5), 74.4 (C-6), 119.2 (C-7), 143.8 (C-8), 44.5 (C-9), 38.3 (C-10), 23.1 (C-11), 40.6 (C-12), 44.8 (C-13), 56.0 (C-14), 24.0 (C-15), 28.9 (C-16), 57.6 (C-17), 12.8 (C-18), 18.8 (C-19), 41.5 (C-20), 21.6 (C-21), 137.0 (C-22), 133.4 (C-23), 44.3 (C-24), 34.4 (C-25), 20.0 (C-26), 20.3 (C-27), 18.0 (C-28).

ACKNOWLEDGMENTS

This work was supported by M.P.I., Rome. Mass spectra were provided by the Servizio di Spettrometria di Massa of the CNR and the University of Naples. The assistance of the staff is gratefully acknowledged.

LITERATURE CITED

1. T. Yasumoto, M. Murata, Y. Oshima, and M. Sano, *Tetrahedron*, **41**, 1019 (1985).
2. M. Murata, M. Sano, T. Iwashita, H. Naoki, and T. Yasumoto, *Agric. Biol. Chem.*, **50**, 2693 (1986).
3. J.E. Bridgeman, P.C. Cherry, A.S. Clegg, J.M. Evans, E.R.H. Jones, A. Kasal, V. Kumar, G.D. Meakins, Y. Morisawa, E.E. Richards, and P.O. Woodgate, *J. Chem. Soc. C*, 250 (1970).
4. W. Arnold, W. Meister, G. Henglert, *Helv. Chim. Acta*, **57**, 1559 (1974).
5. R.F. Zürcher, *Helv. Chim. Acta*, **46**, 2054 (1963).
6. A. Windhaus and A. Luttringhaus, *Justus Liebigs Ann. Chem.*, **481**, 119 (1930).
7. J.L. Dunn, I.M. Heilbron, R.F. Phipers, K.M. Samant, and F.S. Spring, *J. Chem. Soc.*, 1576 (1934).
8. D.P. Michaud, N.T. Nashed, and D.M. Jerina, *J. Org. Chem.*, **50**, 1835 (1985).
9. F. Khuong-Huu, H. Sangare, V.M. Chart, A. Bekaert, M. Devys, M. Barbier, and G. Lukacs, *Tetrahedron Lett.*, 1787 (1975).
10. Y. Yamada, S. Suzuki, K. Iguchi, M. Kikuchi, Y. Tsukitani, H. Moriai, and H. Nakanishi, *Chem. Pharm. Bull.*, **28**, 473 (1980).
11. B.M. Jagodzinska, J.S. Trimmer, W. Fenical, and C. Djerassi, *J. Org. Chem.*, **50**, 1435 (1985).
12. I. Rubistein, L.J. Goad, D.H. Clague, and L.J. Mulheirn, *Phytochemistry*, **15**, 195 (1976).
13. J. Valisolalao, B. Luu, and G. Ourisson, *Tetrahedron*, **39**, 2779 (1983).

14. P. Ceccherelli, F. Fringuelli, G.F. Madruzza, and M. Riboldi, *Phytochemistry*, **14**, 1434 (1975).
15. L.J. Goad, in: "Marine Natural Products: Chemical and Biological Perspectives." Ed. by P.J. Scheuer, Academic Press, New York, San Francisco, and London, 1978, Vol. II, p. 75.
16. M. Kita and Y. Toyoma, *Nippon Kagaku Zasshi*, **81**, 485 (1960).
17. M. Kobayashi and H. Mitsuhashi, *Steroids*, **26**, 605 (1975).
18. A. Malorni, L. Minale, and R. Riccio, *Nouveau Journal de Chimie*, **2**, 351 (1978).

Received 7 March 1988

American Society of Pharmacognosy Awards and Grants

To stimulate interest in all phases of natural product research, the American Society of Pharmacognosy is offering the following awards and grants:

1. Research Starter Grants:

Members of the Society who are within the first five years of assuming their first professional position are eligible for a grant of $2000 to $5000 to aid in their research. These grants will not provide overhead monies to the grantee institution. Applications are due February 1, 1989.

2. Travel Grants for Active Members:

Members of the Society who are within the first five years of assuming their first professional position are eligible for a grant of $300 to $500 to enable them to present a paper at the annual meeting of the Society. Applications are due May 1, 1989.

3. Travel Grants for Graduate Students:

Graduate students under supervision of a Society member are eligible for a grant of $300 to $500 to enable them to present a paper at the annual meeting of the Society. Applications are due May 1, 1989.

4. Undergraduate Research Awards:

Undergraduate students interested in investigating a career in natural product research are eligible for a research award to study under the direction of a Society Member on the faculty of an American college or university. These awards ($2000 to the student and $500 to the advisor) do not provide overhead monies to the grantee institution. The research project is to be accomplished in a summer during the student's academic program leading to the B.S. or Pharm.D. degrees. Applications are due February 1, 1989.

Address inquiries and requests for application information to:

Chris M. Ireland, Ph.D.
Chairman, Awards Committee
American Society of Pharmacognosy
College of Pharmacy
University of Utah
Salt Lake City, UT 84112