Journal of Natural Products
Vol. 50, No. 6, pp. 1171-1173, Nov-Dec 1987

HOMOLOG OF KELLETININ I FROM THE MEDITERRANEAN PROSOBRANCH *BUCCINULUM CORNEUM*

GUIDO CIMINO, SALVATORE DE STEFANO, and GIUSEPPE STRAZZULLO

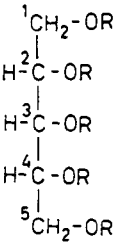
Istituto per la Chimica di Molecole di Interesse Biologico del CNR, via Toiano no. 6, 80072, Arco Felice, Napoli, Italy

Gastropod prosobranchs are generally protected against predators by a hard shell; however, in gastropod opisthobranchs there is an evolutionary trend toward the loss of the shell. The alternative defense strategies exhibited by these mollusks have been the object of numerous chemical studies (1,2). In our continuing effort (3,4) to investigate the chemical defense mechanism of Mediterranean opisthobranchs, we have been attracted by the observation that the shell of the Buccinidae prosobranch *Buccinulum corneum* L. is always devoid of encrustations. Chemical characterization of the metabolic contents of *B. corneum* has led to a new compound [**1**] closely related to kelletinins I and II [**2**,**3**], two antibacterial metabolites previously found (5) in the prosobranch *Kelletia kelletii*.

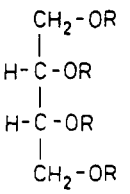
The Et_2O solubles from the Me_2CO extract of the deshelled mollusks were chromatographed on a Si gel column and gave the known kelletinin I [**2**] along with a more polar compound named kelletinin A [**1**].

Kelletinin A [**1**], $C_{40}H_{32}O_{15}$, determined by fabms in conjunction with nmr data, displays spectroscopic evidence very similar to those recorded for **2**. The 500 MHz ^{1}H-nmr spectrum suggests the presence of three types of nearly equivalent *para*-disubstituted benzene rings in a ratio of 2:2:1. The aliphatic protons resonate at δ 5.90, 5.76, and 4.50 in a ratio of 1:2:4. Homonuclear decoupling experiments indicated that the protons at δ 5.76 are coupled with all the other aliphatic protons, whereas that at δ 5.90 is coupled only with those at δ 5.76. These data suggest that **1** should be a penta-*p*-hydroxybenzoate of one of the following four possible stereoisomeric pentitols: D- and L-arabinitol [**4**], ribitol [**5**], and xylitol [**6**].

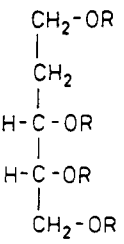
As the natural compound is not optically active, it should be a derivative of **5** or **6**. Upon treating **5** and **6** with *p*-anisoyl chloride, only the pentaester of **5** showed ^{1}H-nmr data identical to those of **1a** obtained by treatment of **1** with an excess of CH_2N_2. Compound **6a** exhibited a ^{1}H-nmr spectrum similar to that of **1a** but with some differences in the



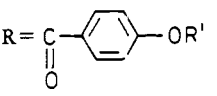
1 and **1a**



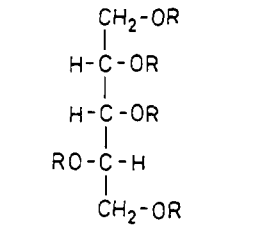
2

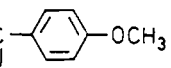


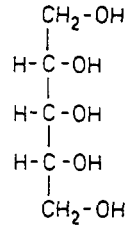
3



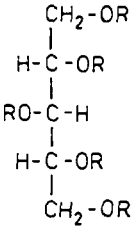
1,**2**,**3** R′=H
1a R′=Me

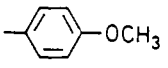


4 R=H
4a R= 



5



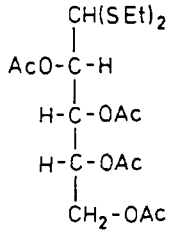
6 R=H
6a R= 

chemical shifts of the aliphatic protons and in the values of the coupling constants. In order to exclude a natural racemic mixture, L-arabinitol [**4**] was esterified, giving **4a**, which showed a more complex ^{1}H-nmr spectrum without symmetric overlaps.

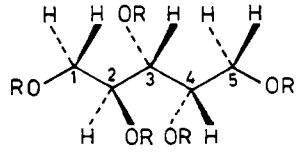
This spectrum showed in the aromatic region two sets of ten overlapped protons at δ 8.03-7.88 and 6.92-6.78, easily attributable to the aromatic protons α to the carbonyl functions and to the methoxy groups, respectively. In the aliphatic field it exhibited, in addition to the signals due to the five methoxy groups, a double doublet (J=3.6 and 7.2 Hz) at δ 6.10 assigned to a methine proton (H-3) coupled with two other protons, resonating as multiplets at δ 5.98 (H-4) and 5.86 (H-2), which in turn were coupled with the protons of the terminal methylenes. The signal at δ 5.86 (H-2) showed couplings with those at δ 4.82 and 4.53 (H-1), while that at δ 5.98 was coupled with those at δ 4.68 and 4.59 (H-5). The assignments were made on the basis of ^{1}H-^{1}H decoupling experiments and by comparison with the ^{1}H-nmr data of **1a**, which showed resonances for the aliphatic chain only at δ 6.11 (H-3), 5.98 (H-2, H-4), 4.84, and 4.53 (H at C-1 and C-5) in full agreement with the symmetric configuration of ribitol [**5**].

The coupling data of H-3 suggest, by comparison (6) with the data reported for tetra-*O*-acetyl-D-arabinose diethyl dithioacetal [**7**], a preponderantly planar "zig-zag" conformation [**a**] for the arabinitol derivative **4a** in $CHCl_3$ solution. In fact, one of the values (7.2 Hz) is sufficiently large to indicate antiparallel disposition between H-3 and H-2, while the small value (3.6 Hz) of $J_{3,4}$ indicates a predominant gauche relationship of H-3 to H-4. Analogously, the coupling data of H-3 in the ^{1}H-nmr spectra of **1a** (t, J=5.6 Hz) and **6a** (t, J=5.5 Hz) suggest that both the compounds adopt "sickle" conformations to avoid the eclipsed 1,3-interactions between O-2 and O-4.

It is likely that, according to what was suggested (5) for kelletinins I and II, kelletinins I and A are also produced de novo by *B. corneum*. In fact, we have observed that specimens of the mollusk, after 3 months in the aquarium, retain kelletinins in amounts comparable to that present in freshly collected animals.



7



a

The origin of the *p*-hydroxybenzoic acid, from diet or by de novo biosynthesis, in marine animals remains an intriguing enigma. Previously (7), 4-hydroxy-3-tetraprenylbenzoic acid has been found among the metabolites of the Mediterranean sponge *Ircinia muscarum*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were measured on a WM 500 Bruker spectrometer (δ ppm/TMS). Mass spectra were taken at 70 eV on an AEI MS 30 mass spectrometer (eims) and on a Kratos MS 50 spectrometer (fabms). Uv spectra were recorded on a Shimatzu-Bausch and Lomb Spectronic 210 apparatus. Ir spectra were obtained on a Perkin-Elmer 257 spectrometer. Column chromatography was carried out on Merck Si gel 60, and tlc was conducted on Merck Si gel F-254 glass plates of 0.25-mm or 0.5-mm thickness.

ANIMAL COLLECTION, EXTRACTION, AND ISOLATION OF KELLETININS A AND I.—Twenty specimens of *B. corneum* were collected in April 1986, in Pozzuoli Bay at a depth of 10 m. A voucher specimen has been deposited in the Mollusca collection of our Institute. Specimens were frozen, deshelled, and extracted with Me_2CO. The Et_2O solubles (1.6 g) from the Me_2CO extract were chromatographed through a Si gel column (light petroleum ether-Et_2O, 3:7). Two main fractions were collected and further purified by a series of chromatographic steps on a Si gel column ($CHCl_3$-MeOH, 95:5) and on semipreparative tlc (Et_2O), giving kelletinin I [**2**] (100 mg; Rf 0.4) and kelletinin A [**1**] (30 mg; Rf 0.3).

Kelletinin A [**1**].—$C_{40}H_{32}O_{15}$, determined by comparative analysis of nmr and ms data; uv λ max (EtOH) 259 (log ε 4.9); ir ν max (Et_2O) 3400-3200, 1728 cm^{-1}; ^{1}H nmr ($CDCl_3$-CD_3OD, 99:1) δ 7.74 (4H, d, J=8.7 Hz), 7.73 (2H, d, J=8.7 Hz), 7.66 (4H, d, J=8.7 Hz), 6.67 (2H, d, J=8.7 Hz), 6.66 (4H, d, J=8.7 Hz), 6.62 (4H, d, J=8.7 Hz), 5.90 (1H, t, J=5.7 Hz), 5.76 (2H, m), 4.67 (2H, dd, J=12 Hz and 3 Hz), 4.37 (2H, dd, J=12 and 6.2 Hz); ^{13}C nmr ($CDCl_3$-CD_3OD, 99:1) δ 166.3, 165.6, 165.2, 162.2, 162.0, 161.7, 132.0, 131.8, 120.4, 120.1, 119.9, 115.1, 114.9, 70.1, 62.3); fabms *m/z* 753 $(M+H)^+$, 615, 479.

Methylation of kelletinin A.—Treatment of **1** with CH_2N_2 gave **1a**. ^{1}H nmr ($CDCl_3$) δ 8.06 (4H, d, J=8.7 Hz), 7.98 (2H, d, J=8.7 Hz), 7.92 (4H, d, 8.7 Hz), 6.89 (6H, d, J=8.7 Hz), 6.83 (4H, d, J=8.7 Hz), 6.11 (1H, t, J=5.6 Hz), 5.98 (2H, m), 4.84 (2H, dd, J=12.1 and 3.6 Hz), 4.53 (2H, dd, J=12.1 Hz and 6.2 Hz), 3.86 (3H), 3.85 (6H), 3.82 (6H); ms *m/z* (%), 822 (M^+, 10%); ir ν max (liquid film) 1719, 1606, 1512, 1257, 1169, 1096, 1026, 766 cm^{-1}; uv λ max (EtOH) 259 (log ε 4.8) nm.

Synthesis of **1a**.—Polyacylation was performed according to Tymiak and Rinehart (5) by dissolving ribitol (3.04 g) in pyridine (3 ml) and adding *p*-anisoyl chloride (0.9 ml) in 6 ml of $CHCl_3$. After 12 h at room temperature with stirring, the mixture was diluted with 0.1 N HCl (100 ml), and the product was extracted into Et_2O. An aliquot (~5%) of the extract by semipreparative tlc ($CHCl_3$) gave 30 mg of a product identical in every aspect to **1a**.

TREATMENT OF XYLITOL [**6**] WITH *p*-ANISOYL CHLORIDE.—Polyacylation of xylitol (304 mg) yielded **6a** (650 mg); ^{1}H nmr ($CDCl_3$) δ 7.98 (2H, d, J=9.1 Hz), 7.95 (4H, d, J=8.9 Hz), 7.92 (4H, d, J=8.8 Hz), 6.86 (2H, d, J=9.1 Hz), 6.85 (4H, d, J=8.9 Hz), 6.81 (4H, d, J=8.8 Hz), 6.19 (1H, t, J=5.5 Hz), 5.91 (2H, m), 4.7 (2H, dd, J=4.5 and 12 Hz), 4.62 (2H, dd, J=5.5 and 12 Hz), 3.81 (15H).

TREATMENT OF L-ARABINITOL [**4**] WITH *p*-ANISOYL CHLORIDE.—Compound **4** (304 mg), by treatment with *p*-anisoyl chloride, gave **4a** (670 mg); ^{1}H nmr ($CDCl_3$) δ 8.03-7.88 (10 H), 6.92-6.78 (10 H), 6.10 (1H, dd, J=3.6 and 7.2 Hz), 5.98 (1H, m), 5.86 (1H, m), 4.82 (1H, dd, J=3.4 and 12.2 Hz), 4.68 (1H, dd, J=4.6 and 12 Hz), 4.59 (1H, dd, J=6.9 and 12.0 Hz), 4.53 (1H, dd, J=6.7 and 12.2 Hz), 3.84 (3H), 3.82 (3H), 3.81 (6H), 3.78 (3H).

ACKNOWLEDGMENTS

Mass spectra were provided by "Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli." The assistance of the staff is gratefully acknowledged.

Thanks are also due to Mr. Antonio Crispino and Mr. Antonio Trabucco for technical assistance and for their perspicacious field observations.

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **1**, 551 (1984).
2. D.J. Faulkner, *Nat. Prod. Rep.*, **3**, 1 (1986).
3. G. Cimino, S. De Rosa, S. De Stefano, and G. Sodano, *Pure Appl. Chem.*, **58**, 375 (1986).
4. G. Cimino, A. Crispino, S. De Stefano, M. Gavagnin, and G. Sodano, *Experientia*, **42**, 1301 (1986).
5. A.A. Tymiak and K.L. Rinehart, Jr., *J. Am. Chem. Soc.*, **105**, 7396 (1983).
6. D. Horton, P.L. Durette, and J.D. Wander, *Ann. N.Y. Acad. Sci.*, **222**, 889 (1973).
7. G. Cimino, S. De Stefano, and L. Minale, *Experientia*, **28**, 1401 (1972).

Received 1 June 1987