Journal of Natural Products
Vol. 50, No. 2, pp. 307-308, Mar-Apr 1987

1,3,7-TRIMETHYLGUANINE FROM THE SPONGE *LATRUNCULIA BREVIS*

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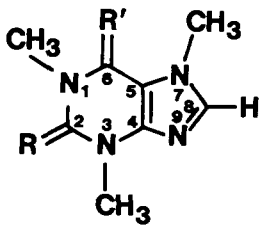
During an investigation of the cytotoxic pigments from New Zealand sponges of the genus *Latrunculia* (family Latrunculiidae) (1), a new purine, 1,3,7-trimethylguanine [**1**], was isolated.

The extract from the sponge *Latrunculia brevis* Ridley and Dendy was partitioned by reverse-phase flash chromatography (2). The purine had been concentrated in one fraction and was further purified by preparative rplc. Ms showed a molecular ion at *m/z* 193 with an accurate mass corresponding to a molecular formula of $C_8H_{11}N_5O$. The ^{1}H-nmr spectrum of the purine was reminiscent of that of caffeine [**2**], with a low-field one proton singlet and three *N*-methyl singlets. The most likely structure seemed to be a trimethyl derivative of either guanine or isoguanine. As both these compounds can be deaminated by heating with HCl (3,4), this reaction was attempted. Conversion of the purine to caffeine [**2**] confirmed the gross structure and demonstrated the methyl substitutions.

The distinction between the two possible structures, 1,3,7-trimethylguanine [**1**] or 1,3,7-trimethylisoguanine [**3**], was achieved by ms. The mass spectra of guanine and its 1-, 3-, and 7-methyl derivatives have been analyzed in detail (5). The initial expulsion of neutral cyanamide fragments, originating from N-1, C-2, and the attached nitrogen, is the most characteristic mode of decomposition of the molecular ions of these compounds. Thus, 1-methylguanine shows ions due to the loss of methyl cyanamide at M-(55,56), which then sequentially lose CO and HCN (5). By a similar retro Diels-Alder mechanism, 1-methylisoguanine loses MeNCO to give a major fragment at M-57 (6). The loss of methyl cyanamide from the molecular ion of the purine, followed by loss of CO then HCN, was confirmed by high-resolution mass measurements (see Experimental Section), showing it to be 1,3,7-trimethylguanine [**1**]. We can find no previous record of this compound, either as a natural or a synthetic product, but various dimethylguanines have been prepared (7-10).



1 R=NH,R'=O
2 R=O, R'=O
3 R=O, R'=NH

1,3,7-Trimethylguanine [**1**] was inactive against the P-388 cell-line in vitro (EC_{50}>10 μg/ml) and had no detectable antiviral or antimicrobial activity.

Several unusual purines have been isolated from sponges. Adenine and 7,9-dimethylguanine betaine (herbipoline) were found in one collection of the sponge species *Geodia gigas* (11,12), whilst another collection contained 1-methyladenine (13). Herbipoline could not be isolated from this second collection, an example of infraspecific variation of sponge metabolites. 1-Methyladenine was also found in *Hymeniacidon sanguinea*, together with 1,9-dimethyl-6-imino-8-oxopurine (14). A series of diterpenes attached to 9-methyladeninium units have been isolated from *Agelas* species (15,16).

The cytotoxic pigments found in the three New Zealand species of the genus

Latrunculia are typified by the imino quinone discorhabdin C (1). No signs of the latrunculins (17) were found in these species.

EXPERIMENTAL

COLLECTION, EXTRACTION, CHROMATOGRAPHY.—The sample of *L. brevis* was dredged from -150 m, off the Otago Peninsula. Identification was by Dr. Graham Fenwick (Department of Chemistry, University of Canterbury) and a voucher specimen (J026-2) retained. The frozen sponge (260 g) was freeze-dried (70 g), powdered and extracted with MeOH-toluene (3:1, 3×500 ml) to give 10.7 g of a dark green tar. This was partitioned by reverse-phase flash chromatography (2). The fraction eluted with H_2O-MeOH (3:1) was largely compound **1** (yellow oil, 75 mg). Si gel tlc (Merck DC-Plastikfolien Kieselgel 60 F_{254}), developed with Et_3N-MeOH-CH_2Cl_2 (0.1:1:4), showed a spot at Rf 0.4 (uv lamp). Rplc analyses involved a 110 mm Brownlee Labs RP-8 Spheri-5 column, with 2 ml/min H_2O (+0.05% TFA)-MeOH (3:2) and uv detection at 210 nm. Further purification was by rplc on a 31 cm×2.5 cm Merck Lobar RP-8 column [H_2O (+0.05% TFA)-MeOH, 1:1] to give a pale yellow oil (62 mg).

1,3,7-TRIMETHYLGUANINE [**1**].—The oil had uv λ max (pH 1, pH 7) 264 nm (log ε 3.3); uv λ max (pH 13) 213 and 288 nm (log ε 3.9 and 3.2); ir ν max (KBr) 3450, 2550, 1730, 1670, 1480, 1210 cm^{-1}; ms *m/z* (%) 194 (14), 193.0965 (M^+; $C_8H_{11}N_5O$ requires 193.0964) (100), 164 (15), 149 (11), 138.0664 ($C_6H_8N_3O$ requires 138.0667) (8), 137 (6), 124 (7), 111 (7), 110.0715 ($C_5H_8N_3$ requires 110.0718) (10), 109 (16), 96 (6), 82.0525 ($C_4H_6N_2$ requires 82.0531), 67 (20); ^{1}H nmr (60 MHz, MeOH-d_4) δ 8.1 (1H, s), 4.0 (3H, s), 3.8 (3H, s), 3.5 (3H, s); ^{13}C nmr (20 MHz, MeOH-d_4) 153.6 (s), 153.1 (s), 148.4 (s), 145.4 (d), 109.8 (s), 34.3 (q), 33.1 (q), 30.0 (q) ppm.

ACID HYDROLYSIS OF 1,3,7-TRIMETHYLGUANINE [**1**].—1,3,7-Trimethylguanine [**1**] (9 mg) was dissolved in HCl (4 ml) and refluxed for 18 h. Removal of the acid and extraction with $CHCl_3$ gave a white solid (3 mg), identified as caffeine [**2**] by comparison with an authentic sample by Si gel tlc (MeOH-CH_2Cl_2, 1:9), rplc coinjection [H_2O (+0.05% TFA)-MeOH, 4:1] and superimposable ^{1}H-nmr (60 MHz, $CDCl_3$) and mass spectra.

ACKNOWLEDGMENTS

This work was supported by the Harbor Branch Oceanographic Institution, Princeton, New Jersey; the Canterbury Medical Research Foundation; and the New Zealand Universities Grants Committee.

LITERATURE CITED

1. N.B. Perry, J.W. Blunt, J.D. McCombs, and M.H.G. Munro, *J. Org. Chem.*, **51**, 5476 (1986).
2. J.W. Blunt, V.L. Calder, G.D. Fenwick, R.J. Lake, J.D. McCombs, M.H.G. Munro, and N.B. Perry, *J. Nat. Prod.*, **50**, 290 (1987).
3. E. Fischer, *Ber.*, **43**, 805 (1910).
4. J.R. Spies, *J. Am. Chem. Soc.*, **61**, 350 (1939).
5. J.M. Rice and G.O. Dudek, *J. Am. Chem. Soc.*, **89**, 2719 (1967).
6. A.F. Cook, R.T. Bartlett, R.P. Gregson, and R.J. Quinn, *J. Org. Chem.*, **45**, 4020 (1980).
7. E. Fischer, *Ber.*, **30**, 2400 (1897).
8. I.M. Ovcharova, L.A. Nikolaeva, E.S. Chapman, and E.S. Golovchinskaya, *Zh. Obshch. Khim.*, **32**, 2010 (1962); *Chem. Abstr.*, **58**, 4567b (1963).
9. I.M. Ovcharova, L.A. Nikolaeva, and E.S. Golovchinskaya, *Khim. Farm. Zh.*, **2**, 18 (1968); *Chem. Abstr.*, **69**, 52099h (1968).
10. K. Ienaga and W. Pfleiderer, *Tetrahedron Lett.*, 1447 (1978).
11. D. Ackermann and P.H. List, *Hoppe-Seyler's Z. Physiol. Chem.*, **308**, 270 (1957).
12. D. Ackermann and P.H. List, *Hoppe-Seyler's Z. Physiol. Chem.*, **318**, 281 (1960).
13. D. Ackermann and P.H. List, *Hoppe-Seyler's Z. Physiol. Chem.*, **323**, 192 (1961).
14. G. Cimino, A. de Giulio, S. de Rosa, R. Puliti, C.A. Mattia, and L. Mazzarella, *J. Nat. Prod.*, **48**, 523 (1985).
15. R.J. Capon and D.J. Faulkner, *J. Am. Chem. Soc.*, **106**, 1819 (1984).
16. H. Wu, H. Nakamura, J. Kobayashi, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett.*, **25**, 3719 (1984).
17. Y. Kashman, A. Groweiss, R. Lidor, D. Blasberger, and S. Carmely, *Tetrahedron*, **41**, 1905 (1986), and references cited therein.

Received 15 August 1986