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A NEW MARINE BETAINE, NORZOOANEMONIN, IN THE GORGONIAN *PSEUDOPTEROGORGIA AMERICANA*

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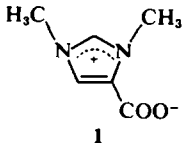
Abstract—The isolation and synthesis of a new betaine are described.

A new betaine (1) has been isolated from the Caribbean gorgonian, *Pseudopterogorgia americana*, from which a number of sesquiterpene hydrocarbons[1] and a seco-gorgosterol derivative[2] have been isolated previously. Compound 1 may be regarded as the nor-derivative of zooanemonin, the homologous dimethylimidazole acetic acid betaine, which was isolated initially by Ackermann[3] from a sea anemone and since encountered[4] in other marine invertebrates.

Norzooanemonin (1) was isolated from the alcohol extract of *P. americana* by ion exchange followed by alumina chromatography. Its structure was deduced from its elemental composition and spectral features, and has been confirmed by synthesis.



1

Norzooanemonin

The betaine's IR spectrum displayed a strong carboxylate ion absorption at 1640 cm^{-1} which shifted to 1710 cm^{-1} upon conversion to the hydrochloride. The heaviest fragment observed in the mass spectrum appeared at *m/e* 96, corresponding to the loss of CO_2 from the molecular ion. The NMR spectrum contained two Me singlets at δ 3·87 and 3·99, and two one proton signals at δ 7·69d ($J = 1·7$ Hz) and 8·63bs. These chemical shifts and coupling constants are very similar to those reported by Caesar and Overberger[5] for methylimidazole methiodide. The signal at δ 8·63 rapidly disappeared when the D_2O solution was made slightly basic with ammonia vapor, typical of the very rapid base catalyzed proton exchange[6] at the 2-position of the dimethylimidazolium nucleus.

Norzooanemonin was synthesized in 85% yield in a single step from imidazole-4-carboxylic acid by methylation with dimethyl sulfate using controlled amounts of aqueous sodium hydroxide to maintain the pH below 9. When this methylation reaction was conducted under the usual conditions of high pH, little or no norzooanemonin could be isolated. Indeed, exposure of norzooanemonin to strong base at room temperature in a separate experiment resulted in its complete decomposition. The m.p. of the synthetic norzooanemonin was not depressed by admixture with the natural product, and the two preparations displayed identical IR and NMR spectra.

In addition to norzooanemonin, the betaine fraction contained a mixture which appeared from its NMR spectrum to consist of trigonelline and possibly homarine (N-methyl nicotinic acid betaine and N-methyl α-picolinic acid betaine, respectively). Each of these betaines is widely distributed in marine invertebrates.[4a,7]

EXPERIMENTAL

M.ps were determined on a Fisher block, unless otherwise indicated, and are uncorrected. IR spectra were taken with a Beckman IR-8 or IR-18A spectrophotometer.

NMR spectra were determined using TMS as an external standard with a Varian T-60 spectrometer and values are reported in δ units.

Isolation of norzooanemonin (*1,3-dimethylimidazole-4-carboxylic acid betaine*). Approximately 3·5 kg of ground cortex of *P. americana* (Florida Keys) which had previously been extracted with both cold and hot hexane was extracted at room temp with two 3 liter batches of 95% EtOH (24 hr each). The filtered EtOH extract was reduced to half its volume and diluted with an equal volume of distilled water. After standing, the ppt was removed by filtration. A small aliquot of the soln was evaporated to dryness, and from the weight of the residue, the soln was estimated to contain approximately 100 g of dissolved solids. The 50% aqueous alcohol soln was then passed through a column of Bio-Rad AG 21 K analytical grade ion exchange resin (hydroxide form; two equivalents based upon an assumed sample equiv wt of 100) which had been washed with 50% aqueous alcohol. The flow rate was adjusted to approximately 0·2 bed volumes per minute.

The effluent soln from the above column was passed directly into a second column of Dowex 50W-XB ion exchange resin (hydrogen form; same exchange capacity as

first column). This column was eluted first with two column volumes of 50% aqueous alcohol, then with 4 liters of 5% ammonium hydroxide.

The ammonium hydroxide soln was evaporated to dryness, leaving 10 g of material which was chromatographed on 400 g of neutral alumina using ethanol-ammonium hydroxide (10:1) as eluent. Ten 25 ml fractions were collected, followed by one 500 ml fraction.

Norzooanemonin (150 mg) was found in the last three small fractions and the single large fraction. It was recrystallized from methanol–acetone and obtained as a colorless solid, m.p. 260–263°; $[\alpha]_D^{25}$ 0°; IR (KBr) 3460, broad, and 1640 cm^{-1} (betaine carboxylate); TLC, R_f 0·63 on alumina in 10:1 EtOH/NH_4OH; negative ninhydrin test; positive Dragendorf test. The NMR spectra discussed earlier were taken in D_2O alone (neutral) and after exposure to NH_3 vapor (basic). (Found: C, 51·30; H, 5·98; N, 19·86. Calcd. for $C_6H_8N_2O_2$: C, 51·42; H, 5·75; N, 19·99%).

The NMR spectrum of the mixture present in the earlier fractions (2 through 5) of the above chromatography indicated the presence of trigonelline and possibly homarine. However, the mixture was not studied further.

Preparation of norzooanemonin hydrochloride. The unstable hydrochloride was prepared by evaporation of a HCl soln of norzooanemonin, and found to melt at 213–217°.

Preparation of norzooanemonin chloraurate. To a soln of 5 mg norzooanemonin in 2 ml of conc HCl were added 10 drops of 30% gold chloride. Within 5 min well formed crystals appeared having a m.p. of 167–169°.

Synthesis of norzooanemonin. To a soln of 4 g (35·8 mmol) imidazole-4-carboxylic acid[a] (m.p. 263°, A. H. Thomas Unimelt) and 1·43 g NaOH (35·8 mmol) in 200 ml water were added 13·6 g (107 mmol) Me_2SO_4. The mixture was stirred at room temp while maintaining the pH between 2 and 9 by periodically adding 10% NaOHaq. After 40 min (all Me_2SO_4 had dissolved) the pH was adjusted to 1 with conc HCl and the soln was heated at 100° for 2 days to hydrolyze the large quantity of $MeHSO_4$ present. After cooling and adjusting the pH to approximately 8 with NaOH, the water was evaporated. An attempt was made to chromatograph the white residue; however a large quantity of $NaMeSO_4$ was still present and could not be separated well. The early fractions, containing the desired product and some $NaMeSO_4$, were combined and dissolved in 150 ml water. The pH of the soln was adjusted to 1 with conc HCl and heated at 100° for 3 days. The soln was cooled, the pH adjusted to 8 with NaOH and the water evaporated at 50°. The residue was slurried with EtOH:NH_4OH (9:1), placed on an alumina column (Fisher A-540, 200 gm, 30 mm × 46 cm) and eluted with the same solvent. The second 100 ml fraction contained 2·87 g (57·5%) pure norzooanemonin; however, later fractions were impure. These were combined and the above hydrolysis and chromatography repeated yielding 1·4 g (28%) more of uncontaminated product. Recrystallization of the first batch from MeOH-acetone gave 2·3 gm white needles, m.p. 250–251°, no depression on admixture with the natural product. The IR and NMR spectra of the synthetic material were identical with those of the natural product.

Treatment of norzooanemonin with base. To 100 mg of norzooanemonin were added a few ml of a strong NaOH soln (pH > 11). The water was evaporated at room temp over a 2–3 hr period and the residue maintained *in vacuo* for 4 days. The NMR spectra in both very basic and slightly basic (pH 8) D_2O indicated complete decomposition.

Stability of norzooanemonin to basic resin. A column of Bio-Rad AG21K which had been converted to the hydroxide form was prepared having a bed volume of approximately 0·9 ml and washed with EtOH:water (1:1). Norzooanemonin (100 mg), dissolved in 0·1 ml EtOH:water (1:1), was placed on the column and rinsed in with two additional 0·1 ml portions. After allowing to stand for 30 min, the column was eluted over a 30 min period with 7 ml EtOH:water (1:1). The solvent was evaporated to dryness leaving 97 mg white residue. The NMR spectrum indicated that only norzoonanemonin was present.

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REFERENCES

[1]A. J. Weinheimer, P. H. Washecheck, D. van der Helm and M. B. Hossain, *Chem. Commun.* 1070 (1968)
[2]E. L. Enwall, D. van der Helm, I. N. Hsu, T. Pattabhiraman, F. J. Schmitz, R. L. Spraggins and A. J. Weinheimer, *Ibid.* 215 (1972)
[3]D. Ackermann, *Hoppe-Seylers Z. physiol. Chem.* **295**, 1 (1953)
[4a]J. H. Welsh and P. B. Prock, *Biol. Bull.* **115**, 551 (1958); [b]D. Ackermann and H. G. List, *Hoppe-Seylers Z. physiol. Chem.* **332**, 198 (1960)
[5]F. Caesar and C. G. Overberger, *J. Org. Chem.* **33**, 2971 (1968)
[6]R. A. Olofson, W. R. Thompson and J. S. Michelman, *J. Am. Chem. Soc.* **86**, 1865 (1964)
[7]J. R. Beers, *Comp. Biochem. Physiol.* **21**, 11 (1967)
[8]R. G. Fargher and F. L. Pyman, *J. Chem. Soc.* **115**, 217 (1919)