Norzooanemonin in the hydroid *Tubularia larynx*[1]

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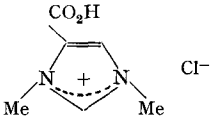
Summary. The hydrochloride of 1,3-dimethyl imidazole-4-carboxylic acid (norzooanemonin), has been isolated from the hydroid *Tubularia larynx* and its structure determined by X-ray analysis.

In the process of purifying the sperm attractant(s) of the hydroid, *Tubularia larynx*, we have isolated trigonelline and homarine (N-methyl nicotinic acid betaine and N-methyl α-picolinic acid betaine, respectively)[2]. While we have not yet identified the sperm attractant, we now report the isolation and X-ray analysis of norzooanemonin hydrochloride (**I**). The betaine from **I** may be regarded as the nor-derivative of zooanemonin, the homologous dimethyl imidazole acetic acid betaine, which was isolated initially by Ackerman[3] from a sea anemone and has since been encountered in other marine invertebrates[4,5].



(**I**)

The crystalline hydrochloride (m.p. 216–219 °C, from methanol: ethanol, 4:1; Weinheimer[6] 213–217 °C) was isolated in low yield (8 mg) by ion exchange and preparative paper chromatography of a water soluble fraction from the original ethanol extract.

Due to the paucity of material an X-ray analysis was done. The crystals of the hydrochloride **I** were orthorhombic with a = 6.403(1), b = 10.721(2), c = 11.632(2) A and d_{calcd} = 1.468 g cm^{-3} for Z = 4 ($C_6H_8N_2O_2 \cdot HCl$, M = 176.60). The systematic absences indicated either space group $P2_1cn$ or Pmcn. The centric space group Pmcn was assumed and the solution and refinement of the structure proceeded satisfactorily. All atoms, except for 4 methyl hydrogen atoms, lie on the mirror plane. The hydrogen atom on O (2) is hydrogen bonded to the chloride ion (O...Cl distance 2.91 A, O–H...Cl angle 176°).

The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered CuKα radiation, θ–2θ scans, pulse height discrimination). The size of the crystal used for data collection was approximately 0.12 × 0.12 × 0.4 mm; the data were corrected for absorption (μ = 38.8 cm^{-1}). Of the 891 accessible reflections for $\theta < 76$ °C, 766 were considered to be observed. The structure was solved by a multiple solution procedure[7] and was refined by full matrix least squares. All hydrogen atoms were located on a difference map calculated after anisotropic refinement of the heavier atoms. In the final refinement, anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R = 0.042 and wR = 0.048 for the 766 observed reflections. There were no peaks greater than ± 0.2 e A^{-3} on the final difference map.

This is the first report of norzooanemonin isolated from a hydroid. It has been isolated previously from the Caribbean gorgonian, *Pseudopterogorgia americana*[6]. In both cases it appears to be accompanied by trigonelline and homarine. Each of these betaines is widely distributed in marine invertebrates[4,5].

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(+)-Abscisic acid, a metabolite of the fungus *Cercospora rosicola*

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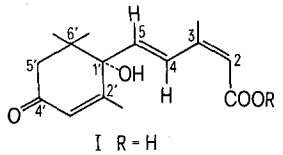
Summary. The first isolation of (+)-abscisic acid from a fungus, *Cercospora rosicola*, is reported.

(+)-Abscisic acid (ABA, **I**) is an important growth regulator in higher plants, and has been isolated from a wide variety of angiosperms and gymnosperms, from a fern, a horsetail, and a moss[2]. It has never been identified in liverworts, algae or fungi[2b]. A single report of abscisic acid in *Penicillium italicum* showed that most probably the fungus had taken the compound up from its host[3]. We wish to report here what we believe to be the first unambiguous evidence of (+)-ABA formed as a secondary metabolite by a fungus. During the screening of the genus *Cercospora* (Deuteromycetes) for secondary metabolites[4], we found that a strain[5] of *Cercospora rosicola* Passerini,

^{13}C chemical shifts (δ) of (+)-ABA

Mult.	δ	Carbon
q	19.1	Me – 2′
q	21.4	Me – 3
q	23.1	2Me – 6′
q	24.3	
s	41.7	C – 6′
t	49.7	C – 5′
s	79.9	C – 1′
d	118.1	C – 2
d	127.0	C – 3′
d	128.3	C – 4
d	136.8	C – 5
s	151.4	C – 2′
s	163.0	C – 3
s	170.9	C – 1
s	198.3	C – 4′

which is frequently found on *Rosa* sp.[6], produces (+)-ABA (6 mg/100 ml maximum) when grown on a potato-agar (PA) medium, at pH 6.5–6.8, 24 °C in the light for 30–40 days. Other culture media, such as yeast-glucose-agar (2% yeast and 20% glucose), malt-agar, oatmeal-agar, are also effective. On stationary potato broth, the average amount of ABA decreases to 2 mg/100ml, whereas no ABA was found when the fungus was grown on Sabouraud maltose agar, GPA, (glucose 30%, peptone 3%, agar), Czapek agar or nutrient agar. Production is much higher than the average amount of ABA in plants[2,7]. The compound was obtained from cultures by direct extraction with AcOEt and chromatography through silica gel with hexane-AcOEt mixtures as eluent.



I R = H
II R = Me

Pure (+)-ABA, $[\alpha]_D^{20} = +384°$ (EtOH, c = 0.23) (Milborrow[8] + 430°) was identified from m.p., UV, IR, NMR and mass spectra of the acid and of the methyl ester (**II**), which appeared identical to those given in the literature[2], and by comparison with a commercial sample of (±)-ABA.

The table lists the ^{13}C NMR resonances ($CDCl_3$, 23.6 MHz) for **I**, which have not been reported so far, and may be useful for biosynthetic studies.

The assignment of the carbons is based on analysis of the undecoupled and off-resonance spectra. In particular, C-3′, Me-2′, C-4, C-5, C-2 and Me-3, have been assigned by selective decoupling for the corresponding ^1H signals[9] and C-2′, C-3 have been distinguished by comparison with data for (+)-S-dehydrovomifoliol[10].

If the production of (+)-ABA, as well as that of auxins by phylloplane fungi[11], occurs also in vitro, this would suggest that fungi may play a role in growth regulation within the ecosystem.

1 Acknowledgments. The authors wish to express appreciation to Prof. Romano Locci for helpful discussions.

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The use of benzylpenicillinacylase from *Escherichia coli* in the resolution of some racemic β-, γ-, δ- and ε-amino-acids

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Summary. The enzymatic hydrolysis of N-phenylacetyl derivatives of racemic amino-acids having the chiral centre removed from the usual α-position is examined. The reaction is found to have different degrees of stereoselectivity. In the case of β-amino-acids and of γ-aminovaleric acid, both enantiomers can be obtained in good yields and high optical purity. S-directed stereochemical preference was found for all the substrates examined.

Preferential hydrolysis of N-acyl derivatives of a variety of L α-amino-acids by enzymes has been exploited for the resolution of racemic mixtures, as well as for the assignment of the absolute configuration.

To our knowledge, no data are available concerning enzymatic hydrolyses of N-acyl derivatives of amino-acids having the chiral centre progressively removed from its usual position α to the carboxyl group. In an effort to explore the synthetic utility, the limitations as well as the rate and the stereoselectivity of the hydrolyses of these substrates, we have examined the enzymatic hydrolysis of the N-phenylacetyl derivatives (N-PA-derivatives) of amino-acids **1–7**.

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