Short Communications

Antiviral agents from a gorgonian, *Eunicella cavolini*[1]

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Summary. The finding of 9-β-D-arabinosyladenine (araA) and of its 3'-O-acetyl derivative in the gorgonian *Eunicella cavolini* is reported. Up to now these substances have not been described as natural compounds but they are well known as potent synthetic antiviral agents. The isolation of spongouridine (araU), 1-β-D-arabinofuranosyluracil, from the same source is also described.

In the course of our search for new polar metabolites from Mediterranean gorgonians[3] we have analyzed the n-butanolic extracts of the gorgonian *Eunicella cavolini*[4]. The investigation allowed us to isolate the 9-β-D-arabinofuranosyladenine, **I**, and its 3'-acetate, **II**, for the first time from a natural marine source, and also spongouridine, **III**; the latter compound was previously found in the sponge *Cryptothetia cripta*.

Material and methods. Fresh material (640 g of *E. cavolini* dry weight after extraction) collected in the bay of Naples was extracted (4 times) with acetone for 3 days. The combined extracts were concentrated and the remaining aqueous solutions were first extracted with diethyl ether (3 times) and later with n-butanol (3 times).

The n-butanolic extracts were taken to dryness under reduced pressure leaving a syrup (3.1 g). This was applied on silica-gel column (Merck 70–230 mesh ASTM) equilibrated with $CHCl_3$-MeOH, 9:1, and was eluted with increasing amounts of methanol to give 2 main fractions: A (79 mg) and B (1 g). Preparative SiO_2-TLC of A in $CHCl_3$-MeOH, 7:3 afforded **II** (50 mg 0.008% melting point 214–215 °C from MeOH; cytotoxicity in vitro 5.0 μg/ml K.B. cells).

The more polar fraction B was acetylated with acetic anhydride and pyridine under reflux for 1 h. The mixture was taken to dryness and applied to a silica-gel column in $CHCl_3$-MeOH, 98:2, that yielded the peracetylated derivative of **I** (250 mg, 0.04%) and the 2',3',5'-tri-O-acetyl derivative of **III** (40 mg, 0.006%); further amounts of tetra and tri-acetyl derivatives of I were also recovered.

Results and discussion. Compound **II**, known as a synthetic product[6], was characterized by spectral data: UV λ_{max}(MeOH) 258 nm (ε = 14,000); IR ν_{max} 1710 cm^{-1}; NMR δ (CD_3OD-$CDCl_3$) 8.45 (1H, s), 8.30 (1H, s), 6.45 (H-1',d, J = 4.3 Hz), 5.30 (H-3',m), 4.35 (H-2', m), 4.20 (H-4',m), 3.95 (2H, m), 2.20 (3H,s); MS 309 (M^+, 2%), 279 (M^+-30, 6%), 266 (M^+-43, 3%), 178 (M^+-101, 15%), 164 (M^+-115, 60%), 136 (72%), 135 (100%), 108 (48%).

The localization of the acetyl group in 3' was suggested by the presence in the MS of the fragments at m/z 279 and 178[7] and by NMR double resonance experiments.

II peracetylated, obtained with acetic anhydride and pyridine (30 min at reflux), was identical to an authentic sample of peracetylated araA.

The spectral data (PMR, IR) of the mixture B excluded the presence of acetyl groups.

After acetylation of B, 2 main products were recovered. The less polar compound was recognized as peracetylated **I** by comparison of the following spectral data with those of an authentic sample of peracetylated 9-β-D-arabinofuranosyladenine. $[\alpha]_D^{CHCl3} = -8°$; UV λ_{max} (MeOH) 268 nm (ε = 7550); IR ν_{max} 2915, 2840, 1720, 1255, 1215, 1035 cm^{-1}; NMR δ ($CDCl_3$) 9.20 (1H, s), 8.40 (1H, s), 6.80 (1H, d, J = 4.5 Hz), 5.50 (2H, m), 4.50 (3H, m), 2.40 (6H, s), 2.28 (3H, s), 2.22 (3H, s), 1.98 (3H, s); MS 477 (M^+, 1%), 435 (M^+ − 42, 6%), 420 (43%), 392 (7.5%), 376 (18%), 350 (6.2%), 316 (13%), 304 (4%), 259 (peracetylated arabinosyl ion, 88.7%), 220 (10%), 135 (100%), 108 (8%).

The second compound showed the following spectral data: $[\alpha]_D^{CHCl3} = +15°$; UV λ_{max} (MeOH) 262 nm (ε = 9200); NMR δ ($CDCl_3$) 7.45 (1H, d, J = 8 Hz), 6.10 (1H, H-1', m), 5.80 (1H, d, J = 8 Hz), 5.45 (2H, H-2' and H-3', m), 4.45 (3H, m), 2.20 (9H, s); MS 370 (M^+, 6.1%), 311 (5%), 310 (7%), 259 (100%), 251 (8%), 250 (9%).

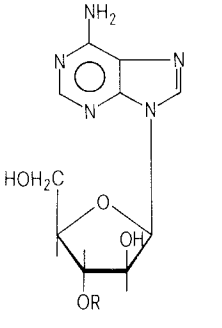
All these data allowed us to suggest the structure **IV**.

The hydrolysis of **IV** with 10% HCl, giving uracil and arabinose, confirmed the proposed structure.

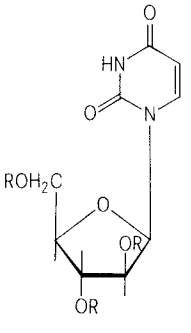
Spongouridine (araU), **III**, which has recently[8] been shown to possess antiviral activities, was previously found together with spongothymidina (araT) in the marine sponge *C. cripta*.

Its resistance[9] to enzymes that rupture the base-sugar bond of uridine strongly stimulated the synthesis of a series of nucleosides[10] with the 'fraudulent' sugar arabinose. Among these, araA[11] exhibited significant antiviral activity[12] against DNA-containing viruses, so that it was the first antiviral drug used as a highly effective agent in the usually fatal herpes encephalitis[13].

Nevertheless its low solubility and the readiness with which it is deaminated were a restraint to the administration of the drug. To overcome these problems some authors synthesized several O-acyl derivatives[6,14] of araA among which the 3'-O-acyl derivatives appeared promising, even though the best properties were observed for the 2'-O-acyl derivatives[6]. Notwithstanding the fact that the 2'-O-acyl derivatives are more effective it seems interesting to us to underline that in nature araA coexists with its 3'-O-acetyl derivative.



I R = H
II R = $COCH_3$



III R = H
IV R = $COCH_3$

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4 The gorgonian collected in the bay of Naples was obtained from the supply Department of the Zoological Station (Naples) to whom the authors express their thanks.
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Molluscicidal and insecticidal activities of isobutylamides isolated from *Fagara macrophylla*

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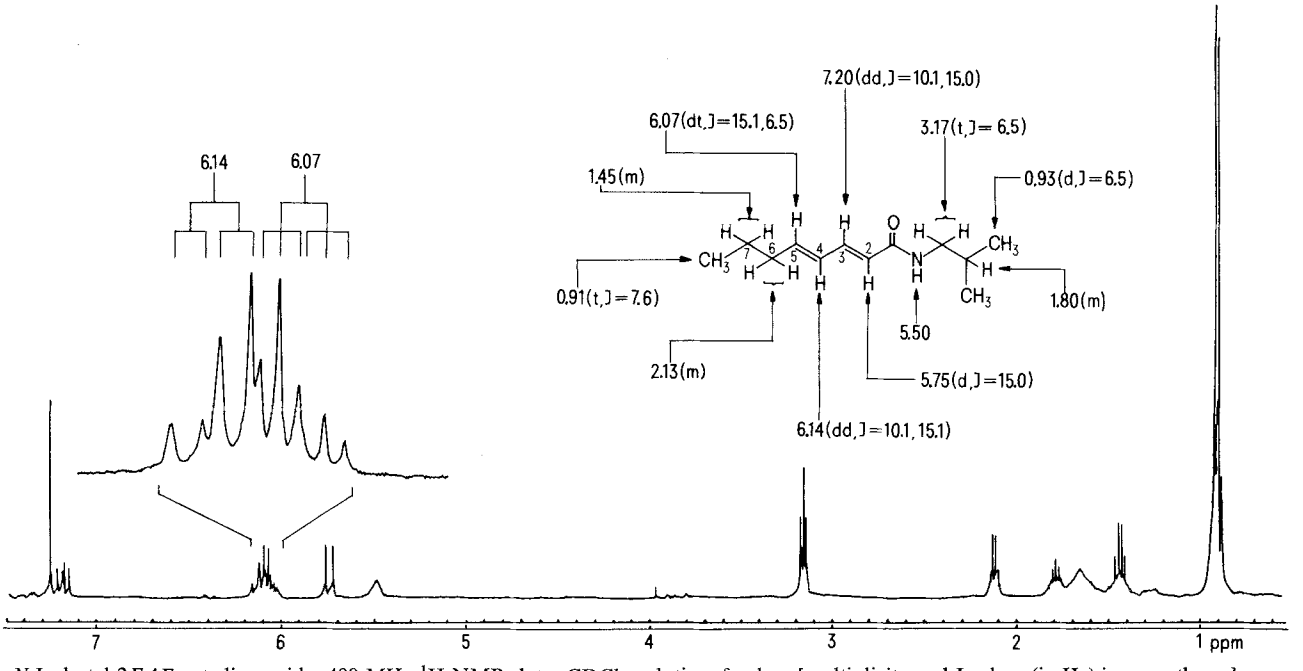
Summary. Five isobutylamides were isolated as insect growth inhibitors and toxicants from *Fagara macrophylla* and identified from their spectroscopic data.

The East African medicinal tree *Fagara macrophylla* (Rutaceae) is known to be relatively free from insect attack. In order to test for chemical factors involved in this observed resistance, extracts of the *F. macrophylla* bark were incorporated into artificial diets optimized for several economically-important agricultural pest insects, pink bollworm *(Pectinophora gossypiella)*, tobacco budworm *(Heliothis virescens)*, corn earworm *(H. zea)* and fall armyworm *(Spodoptera frugiperda)*[2,3]. This led to the isolation of 5 insect growth inhibitors and/or toxicants which have been identified as fagaramide [*N*-isobutyl-3-(3,4-methylenedioxyphenyl)-2*E*-propenamide] (**1**), piperlonguimine [*N*-isobutyl-5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienamide] (**2**)[4,5], 4,5-dihydropiperlongumine [*N*-isobutyl-5-(3,4-methylenedioxyphenyl)-2*E*-pentenamide] (**3**)[6], pellitorine (*N*-isobutyl-2*E*,4*E*-decadienamide) (**4**)[7], and *N*-isobutyl-2*E*,4*E*-octadienamide (**5**)[8] based on spectroscopic data.

The most abundant of the isolated amides was fagaramide **1**[9]. The structure of fagaramide has long been known[10], but the geometry of its side chain double bond has not been clearly established. This has now been confirmed as *trans* based on the large coupling constant (16 Hz) in the 400 MHz ^{1}H-NMR spectrum.

The ^{1}H-NMR spectra were more important in assigning the stereochemistry of conjugated double bond in **2**, **4** and **5**. For example, assignment of the geometry of *N*-isobutyl-2*E*,4*E*-octadienamide **5** was achieved as follow. The ^{1}H-NMR spectrum of **5** is reproduced in the figure along with an expanded representation of a part of the olefinic proton region. Irradiation of the 3-H doublet of doublets at 7.20



N-Isobutyl-2*E*,4*E*-octadienamide, 400 MHz ^{1}H-NMR data, $CDCl_3$ solution, δ values [multiplicity and J values (in Hz) in parentheses].