A naturally-occurring analog of methylthioadenosine (MTA) from the nudibranch mollusc *Doris verrucosa*

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Summary. 9-[5'-deoxy-5'-(methylthio)-β-D-xylofuranosyl]-adenine, the first naturally occurring analog of methylthioadenosine (MTA), was isolated from the marine nudibranch mollusc *Doris verrucosa*. This finding provides indirect evidence for the existence in nature of analogs of S-adenosyl-L-methionine (AdoMet), the ubiquitous naturally-occurring biological methyl donor.

Key words. 9-[5'-deoxy-5'-(methylthio)-β-D-xylofuranosyl]-adenine; methylthioadenosine; MTA; S-adenosyl-L-methionine; AdoMet; nudibranches; *Doris verrucosa*.

S-Adenosyl-L-methionine (AdoMet) (**1**) is the ubiquitous naturally-occurring biological methyl donor which functions also as a propylamine donor for polyamine synthesis.

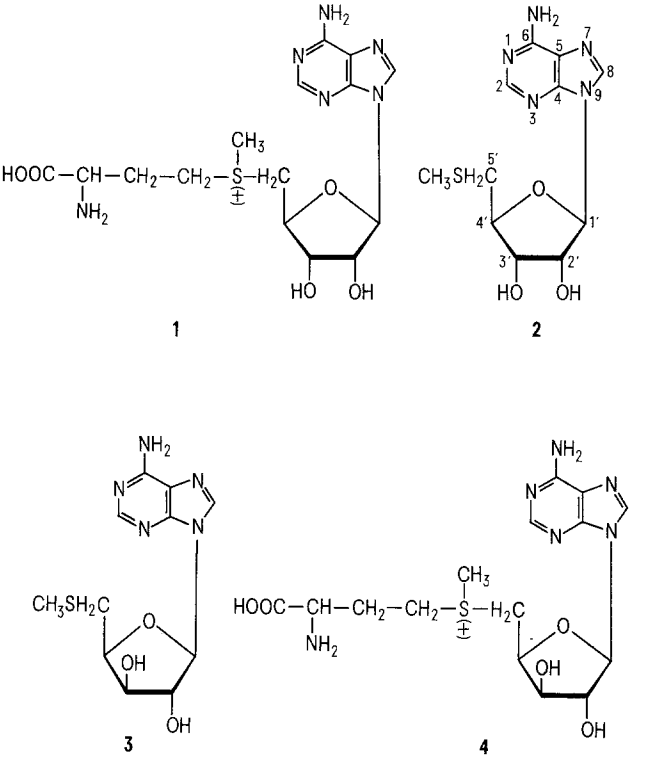
AdoMet is cleaved either chemically[1] or enzymatically[2] to 9-[5'deoxy-5'-(methylthio)-β-D-ribofuranosyl] adenine (methylthioadenosine; MTA) (**2**), a metabolite which is attracting increased attention for its highly diverse regulatory functions[3].

We wish to report on the isolation and identification of 9-[5'-deoxy-5'-(methylthio)-β-D-xylofuranosy]] adenine (**3**), an analog of MTA in which the deoxyribofuranosyl moiety is replaced by a deoxyxylofuranosyl residue.

During studies on the chemical defense mechanisms of nudibranch molluscs[4] we have examined the Mediterranean nudibranch[5] *Doris verrucosa* Cuvier. Approximately 300 specimens of *D. verrucosa* (average length 3 cm) were dissected and the digestive glands and the mantles were separately extracted with acetone. Both extracts were evaporated at reduced pressure and the residual water was extracted sequentially with diethyl ether and n-butanol. The ethereal extract of the mantles contained a complex mixture of diterpenoid glycerides[6] which could account for the chemical defense of these molluscs, while both n-butanol extracts contained the major compound (**3**) which was isolated by fractionation on a column of Sephadex LH-20 using methanol as eluant. 30 mg of pure **3**, $[\alpha]_D^{25}$-270° (c = 0.39; pyridine) was isolated from the mantles and 35 mg from the digestive glands, raising the yield to about 0.2 mg per animal.

High resolution mass spectrometry[7] indicated a molecular formula of $C_{11}H_{15}N_5SO_3$. The fragment ions were the same as those occurring in the mass spectrum of synthetic MTA (**2**) with the exception of a prominent ion at m/z 250 (M^+-SCH_3) which is absent from the previously reported[8] mass spectrum of **2**. Compound **3** had an UV absorption identical to that of **2** (λ_{max} 260 nm at pH 7 and 257 nm at pH 2) indicating the presence of a 9-substituted adenine nucleus.

Furthermore, compound **3** was easily separated from MTA (**2**) on silica gel thin-layer chromatography (chloroform-methanol 8:2; **3**, R_f 0.70; **2**, R_f 0.65). These data suggest that **3** is stereoisomeric with **2**. This view was further corroborated by the comparison of the proton nuclear magnetic resonance (^{1}H-NMR) spectra of **2** and **3** (table). The major distinguishing features are the chemical shifts and the coupling constants of the pentose moieties.



Proton NMR spectra of 9-[5'-deoxy-5'-(methylthio)-β-D-ribofuranosyl]adenine (**2**)[a] and 9-[5'-deoxy-5'-(methylthio)-β-D-xylofuranosyl] adenine (**3**) in dimethylsulfoxide-d_6 (DMSO-d_6). The spectra were obtained on a Brucker 250 MHz FT instrument; DMSO-d_6 was used as internal standard at δ 2.49. Assignments were aided by homonuclear decouplings and D_2O exchange at the OH protons

Position	Compound **2** Chemical shift δ (ppm)	Multiplicity,[b] relative area	Coupling constants (Hz)	Compound **3** Chemical shift δ (ppm)	Multiplicity,[b] relative area	Coupling constants (Hz)
C-2	8.14	s, 1H		8.14	s, 1H	
C-8	8.35	s, 1H		8.24	s, 1H	
C-1'	5.88	d, 1H	$J_{1'-2'} = 5.8$	5.86	d, 1H	$J_{1'-2'} = 1.4$
C-2'	4.74	aq, 1H	$J_{2'-3'} = 5.1$	4.30	m, 1H	$J_{3'-2'} \cong 0$
C-3'	4.17	aq, 1H	$J_{3'-4'} = 3.7$	4.00	m, 1H	$J_{3'-4'} = 3.2$
C-4'	4.01	m, 1H		4.27	dt, 1H	$J_{4'-5'} = 6.8$
			$J_{5'A-5'B} = 13.9$			
C-5'	2.87 5'A	dd, 1H	$J_{5'A-4'} = 5.9$	2.86	dd, 1H	$J_{5'A-5'B} = 13.5$
	2.74 5'B	dd, 1H	$J_{5'B-4'} = 6.9$	2.74	dd, 1H	$J_{5'A-4'} = J_{5'B-4'} = 6.8$
S-CH_3	2.04	s, 3H		2.10	s, 3H	
2'-O*H*	5.49	d, 1H	J = 6.1	6.00	d, 1H	J = 3.9
3'-O*H*	5.31	d, 1H	J = 4.9	6.11	d, 1H	J = 5.5
6-NH_2	7.29	s, 2H		7.34	s, 2H	

[a]Purchased from Sigma. [b]Singlet, s; doublet, d; doublet of doublets, dd; doublet of triplets, dt; apparent quartet, aq; multiplet, m.

A literature search revealed that the ^{1}H-NMR data of **3** correspond to those reported[9] for synthetic 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl] adenine. A direct comparison of the ^{1}H-NMR spectra of natural and synthetic **3**[10] confirmed the identification.

From a biogenetic point of view compound **3** could reasonably be derived by cleavage of the analog of AdoMet carrying a deoxyxylofuranosyl moiety (**4**), in a fashion which is similar to the origin of MTA from AdoMet. The interest in the analogs and derivatives of AdoMet lies mainly in the possibility of modification and control of the transmethylation reactions. Since it is the first time that a MTA analog has been detected in nature, the conjecture just cited suggests that analogs of AdoMet might also occur in living systems.

To our knowledge, compound **3** also constitutes the first naturally-occurring purine carrying a xylose derivative substituent.

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0014-4754/86/11/121301-02$1.50 + 0.20/0

Naturally-occurring crystals of photocarcinogenic furocoumarins on the surface of parsnip roots sold as food

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Summary. Normal levels of total furocoumarins in fresh parsnip roots are as high as 96 ppm (wet weight). In older 'spoiled' and diseased parsnips freely available in grocery stores these values may be increased by 2500%. So high are the amounts in some instances, that mixed crystals of furocoumarins can be detected on the surfaces of the parsnip roots by both conventional low powered microscopy and SEM. The principal crystal form was angelicin but the presence of psoralen, 5-MOP and 8-MOP was confirmed by HPLC, TLC, photobiological assay and analysis of mass spectra.
Key words. Parsnip; furocoumarins; photocarcinogenesis; 5-methoxypsoralen; 8-methoxypsoralen; psoralen; angelicin; photosensitivity.

A number of plants belonging to the family Umbelliferae produce linear and angular furocoumarins (fig. 1) of which psoralen may be taken as representative of the former and angelicin of the latter. Increased levels of these molecules are probably associated with the phytoalexin response to stress[1,2] even though levels of furocoumarins may be normally high in many Rutaceae and Umbelliferae. Linear furocoumarins such as psoralen and 5-methoxypsoralen (5-MOP) can, in the presence of near ultraviolet (UV), produce both DNA monoadducts and the more lethal DNA-DNA interstrand crosslinks. Angular furocoumarins such as angelicin, under normal circumstances, form DNA monoadducts only[3]. When excited by near UV radiation (300–380 mn) furocoumarins produce lethal, mutagenic and clastogenic effects in numerous animal and human cells[4,5]. Skin cancer in animals[6,7] and probably in man[8,9] are known consequences of photosensitization with furocoumarins.

Skin photosensitivity after contact with diseased celery, a plant belonging to the Umbelliferae, has been established[10] to result from the large amounts of three furocoumarins: psoralen, 5-methoxypsoralen (5-MOP) and 8-methoxypsoralen (8-MOP). Ivie et al.[11], have drawn attention to the presence of furocoumarins as natural toxicants in parsnips. Total concentrations as high as 40 ppm were reported and the relevance of these findings to human consumption has been discussed by Ames[12]. Numerous reports on the adverse effects of contact with parsnips have appeared in the literature including several in which troops had been suspected of contact with mustard gas, so severe were the skin reactions[13].

Methods. The HPLC techniques for the analysis of furocoumarins in plants together with the ultrasensitive biological assays following two dimensional TLC have been described previously[14–16]. Parsnips were obtained in Victoria, British Columbia, Canada.

Results and discussion. During the course of previous studies on celery[4,14] we were surprised to find, on occasions, very high levels of furocoumarins in some parsnip samples. These were levels far in excess of those reported by Ivie et al.[11] and greater even than the levels of 8-MOP in parsnip pieces infected with fungus and grown in vitro[17]. Examination of several 'spoiled' parsnip roots with a low power dissection microscope revealed the presence of what appeared to be small pieces of fungal mycelium but which,

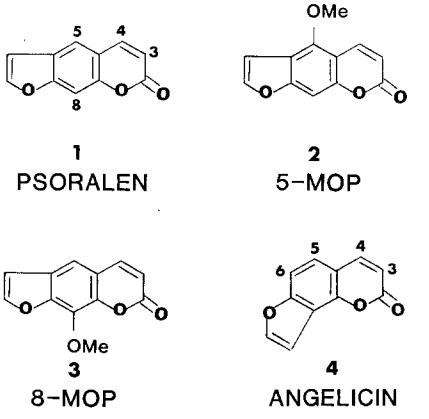

Figure 1. Formulae of photosensitizing furocoumarins found in parsnip root.