ASNIPYRONES A AND B, TWO NOVEL METABOLITES FROM ASPERGILLUS NIGER

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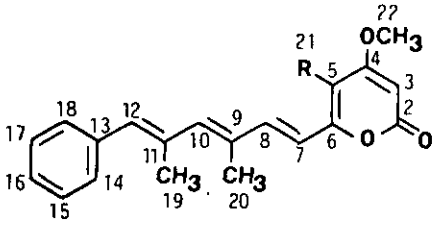
Abstract - Two bright yellow coloured metabolites, named asnipyrone A $C_{21}H_{22}O_3$ and asnipyrone B $C_{20}H_{20}O_3$, have been isolated from Aspergillus niger strain DSM 2182[2]. On the basis of chemical reactions and spectroscopical data their structures were derived as the phenyltrienyl-α-pyrones (1) and (2).

The fungus Aspergillus niger, well-known from its deeply black mycelium, needs special attention as it spreads aggressively on numerous kinds of food and feed and is also applied for large scale industrial fermentations. Two groups of toxic metabolites have already been isolated from it[1]. Our investigation on mycelia from Aspergillus niger DSM 2182[2] led, besides ergosterol and heminigerone (3)[3], to the isolation of two hitherto unknown metabolites, the asnipyrones A and B, for which the structures (1) and (2) could be determined.

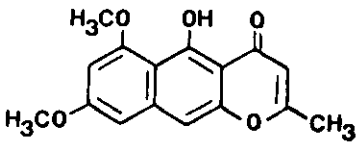
The structure of the trienylpyrone part of the new metabolites is strikingly similar to that of citreoviridin A (4)[4,5], a highly potent mycotoxin from Penicillium viride[4] and Aspergillus terreus[5,6], which interferes with the mitochondrial ATP synthesis and causes the epidemic mycotoxicosis cardiac beriberi[7].

Asnipyrone A (1) crystallizes from ethanol as yellow needles, and its high resolution mass spectrum reveals the molecular formula $C_{21}H_{22}O_3$. The uv/vis spectrum shows some analogy to citreoviridin A (4)[8]. The ir absorptions at 1670, 1626, and 1538 cm^{-1} are consistent with the presence of an α-pyrone unit[8]. The ^1H and ^{13}C nmr data (Table 1) indicated that asnipyrone

A (1) contains three methyl groups, one methoxy group, five aromatic protons and five vinylic protons.



1: R = CH_3
2: R = H



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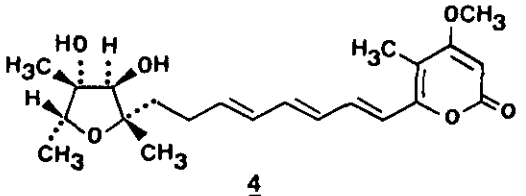

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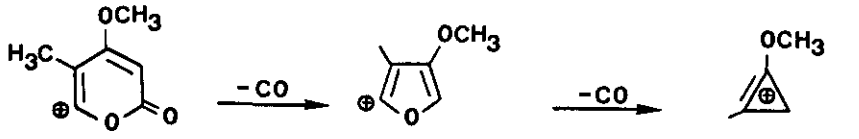
Comparison of the nmr spectra of citreoviridin (4) and asnipyrone A (1) provided a clear evidence for the presence of a 4-methoxy-5-methyl-α-pyrone unit as a partial structure in the latter.



m/z 139(125) m/z 111(97) m/z 83(69)

Scheme 1. Ms fragmentation of α-pyrone units from asnipyrones A (1) and B (2). Data of (2) in brackets. M/z values 139(125) are base peaks (intensities 100 %).

The analysis of fragment ions in its mass spectrum also tallies with this assumption (Scheme 1). Furthermore, the mass spectrum of asnipyrone A (1) shows a benzyl and a phenyl cation peak at m/z 91 and 77 respectively. After $KMnO_4$ oxidation of (1) benzoic acid was obtained. These results proved that a benzene ring exists in this metabolite. Hexahydroasnipyrone A $C_{21}H_{28}O_3$ was obtained as a colourless oil after hydrogenation of (1) with palladium catalyst in methanol, indicating the presence of three double bonds, which can only be accomodated between the α-pyrone and the benzene ring.

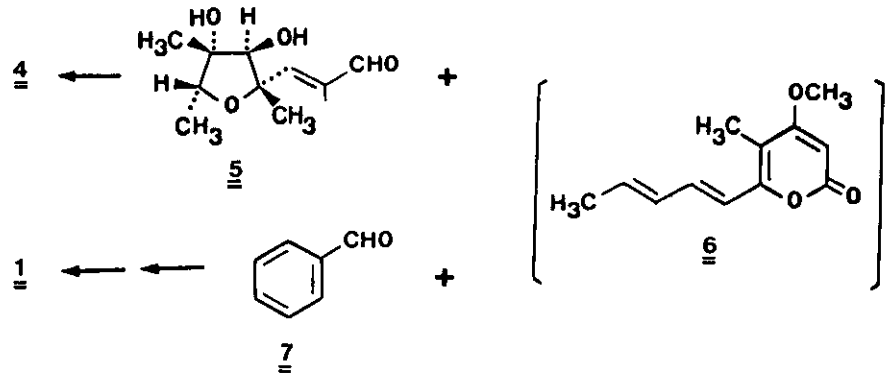
In the ^1H nmr spectrum (Table 1) of asnipyrone A (1) signals for two mutually coupled protons were observed at 6.10 and 7.29 (J = 15.6 Hz). These should be the two protons at C-7 and C-8. Two methyl groups at 2.07 and 2.10 ppm showed long range coupling with two proton signals at 6.54 and 6.40 respectively. Except for this long range interaction these two protons showed no coupling with other protons. Therefore, the two methyl groups and two protons should be linked to C-9, C-11, C-10, C-12. Thus the structure of asnipyrone A is deduced as (1).

Table 1. ^1H and ^{13}C nmr data (δ, $CDCl_3$) for the asnipyrones A (1) and B (2).

Pos. No.	asnipyrone A 1 ^{13}C	^1H	J/Hz	asnipyrone B 2 ^{13}C	^1H	J/Hz
2	164.77			164.07		
3	95.21 d	6.09 1H, s		88.29 d	5.89 1H, d	2.0
4	165.80 s			171.16 s		
5	102.72 s			100.72 d	5.42 1H, d	2.0
6	157.91 s			159.29 s		
7	117.81 d	6.10 1H, d	15.6	111.46 d	6.12 1H, d	15.6
8	142.05 d	7.29 1H, d	15.6	142.38 d	7.23 1H, d	15.6
9	133.14 s			133.11 s		
10	140.85 d	6.54 1H, s, br		141.64 d	6.57 1H, s, br	
11	134.96 s			134.96 s		
12	132.96 d	6.40 1H, s, br		133.02 d	6.44 1H, s, br	
13	137.33 s			137.39 s		
14	126.75 d	7.26-7.36 = 5H		126.81 d	7.18-7.41 = 5H	
15	129.09 d			129.15 d		
16	128.07 d			128.13 d		
17	129.10 d			129.15 d		
18	126.75 d			126.81 d		
19	18.72 q	2.10 3H, d	1.0	18.75 q	2.11 3H, d	1.0
20	13.85 q	2.07 3H, d	1.0	13.88 q	2.08 3H, d	1.0
21	8.69 q	1.96 3H, s		-	-	
22	56.09 q	3.89 3H, s		55.88 q	3.81 3H, s	

Asnipyrone B (2) crystallizes from methanol/methylene chloride as yellow prisms. Its high resolution mass spectrum indicated the molecular formula $C_{20}H_{20}O_3$. From a comparison of the ^1H and ^{13}C nmr spectra (Table 1) it could be derived that asnipyrone B differs from asnipyrone A (1) by the absence of a methyl group at C-5. This was indicated by the fact that the proton at C-5 (5.42) gives a long range coupling (J = 2.0 Hz) with the C-3 proton (5.89). The mass spectrum also supported this structure (Scheme 1). The longest wave length uv/vis absorption of asnipyrone B at 367 nm is shifted by 11 nm to shorter wave lengths compared with that of (1). In agreement with all these results the structure of asnipyrone B can be represented by (2).

From the structures of the asnipyrones A (1) and B (2) a close relationship to the well established biosynthesis of the mycotoxin citreoviridin (4)[5,9] is suggested. According to comprehensive feeding experiments citreoviridin (4) is constructed from nine acetate units possibly via a polyketide chain, substituted with methyl groups from methionine.



Recently the isolation of the C_{11} aldehyde citreoviral (5) and (4) from Penicillium citreoviride and further metabolites was reported[10]. It seems possble that (5) might act as a biogenetic precursor for (4) by condensation with the α-pyrone building block (6). Analogous condensation of (6) with the ubiquitous metabolite benzaldehyde, and subsequent introduction of two methyl groups would lead to asnipyrone A (1).

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EXPERIMENTAL

Mps (corrected): Kofler hot-stage apparatus. - Uv/vis: Perkin-Elmer 551. - Ir: Perkin-Elmer 298. - Ms: Finnigan-MAT 312, and Varian MAT CH7. - ^{1}H Nmr: Bruker WM 300, TMS as an internal standard. - ^{13}C Nmr: Bruker WH 90 (22.63 MHz). - TLC: Merck silica gel 60 F 254 on aluminum sheets. - Column chromatography: Merck silica gel, particle size 0.063 - 0.2 mm.

Isolation of metabolites from Aspergillus niger.

a) Preparation of mycelia: 127.8 g of $NaNO_3$, 63.9 g of KH_2PO_4, 32.0 g of KCl, 32.0 g of $MgSO_4$-. 7 H_2O, 0.64 g of $FeSO_4$, 3.195 kg of D-glucose, and 63.9 g of yeast were dissolved in 63.900 l of dist. water and distributed into 320 (1000 ml) flasks. After sterilization, each flask with culture medium was inoculated with Aspergillus niger strain DSM 2182[2] and grown at 25° C for three weeks. Subsequently 2 ml of toluene was added to each flask, the mycelia were collected and freeze-dried, yielding 1014 g of dried mycelia.

b) Extraction and isolation: The dry mycelia were extracted four times with 6 l of cold methanol, and the combined extracts were concentrated to 200 ml. Addition of 2000 ml of water to the concentrate brought about the precipitation of a brown solid, which was separated by filtration and dissolved in EtOAc. After filtration the solution was evaporated to give 56.6 g of residue. The residue was subjected to column chromatography on silica gel (1:500 w/w) by elution with cyclohexane/EtOAc (3:1 v/v). 180 fractions (ca. 20 ml each fraction) yielding the metabolites as shown below were obtained.

1) Fractions 10 - 21: ergosterol

2) Fractions 22 - 60: 302 mg of asnipyrone A (1)

3) Fractions 81 -120: 120 mg of asnipyrone B (2)

4) Fractions 123 -180: 7 mg of heminigerone (3).

Ergosterol: Colourless needles mp 149 - 151 °C.

Heminigerone (3): Yellow needles, mp 217-219 °C (Lit.[3] 178-180 °C). - Ir and TLC were identical with authentic sample. Ms Calcd for $C_{16}H_{14}O_5$: 286.0841. Found m/z: 286.0840.

Asnipyrone A (1): Yellow needles, mp 161 - 163 °C. Uv/vis (MeOH): λ_{max} (lg ε) 240 (4.27), 290 (4.28), 378 nm (4.50). Ir (KBr): 2970, 2930, 2840 (CH), 1670, 1626, 1538 cm^{-1} (α - Pyrone). ^{1}H and ^{13}C nmr: table 1. Ms: m/z 322 (M^+), 307 (M - CH_3), 294 (M - CO), 279 (294 - CH_3), 139 (100 %, α-pyrone unit, scheme 1), 111 and 69 (scheme 1). Ms calcd for $C_{21}H_{22}O_3$: 322.1569. Found: m/z 322.1573.

Hydrogenation of asnipyrone A: 50 mg of (1) in 50 ml of MeOH were hydrogenated with Pd(10 %)/charcoal catalyst until the solution became colourless. Filtration and evaporation

yielded 33 mg of hexahydroasnipyrone A as a colourless oil. Ms: m/z 328 (M^+), 237 (M^+ - 91), 167 (100 %), 91 (benzyl).

Oxidation of asnipyrone A: To a stirring solution of 20 mg of (1) in 1.5 ml of pyridine 52 mg of pulverized $KMnO_4$ was added at 0 °C. After 1 h the mixture was adjusted to pH 4-5 with diluted H_2SO_4 and treated with $NaHSO_3$ to dissolve MnO_2. On extraction with ether after acidifying to pH 2, 3 mg of a crystalline solid, mp 107 - 112 °C was obtained. Ir and TLC were identical with benzoic acid.

Asnipyrone B (2): Yellow prisms, mp 158 - 160 °C. Uv/vis (MeOH): λ_{max} (lg ε) 235 (4.22), 265 (4.19), 367 nm (4.53). Ir (KBr): 3060, 2940 (CH), 1705, 1630, 1595, 1538 cm^{-1} (α-Pyrone). ^1H and ^{13}C nmr: Table 1. Ms: m/z 308 (M^+), 293 (M - CH_3), 125 (100 %, pyrone unit, scheme 1), 97 and 69 s. scheme 1. Ms calcd. for $C_{20}H_{20}O_3$: 308.1412. Found: m/z 308.1412.

Hydrogenation of asnipyrone B: 30 mg of (2) were hydrogenated as described for (1) yielding 10 mg of hexahydroasnipyrone B as a colourless oil. Ms: m/z 314 (M^+), 223 (M - 91), 153 (100 %), 91 (benzyl).

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