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LUNATOIC ACID A AND B, AVERSION FACTOR AND ITS RELATED METABOLITE OF COCHLIOBOLUS LUNATA

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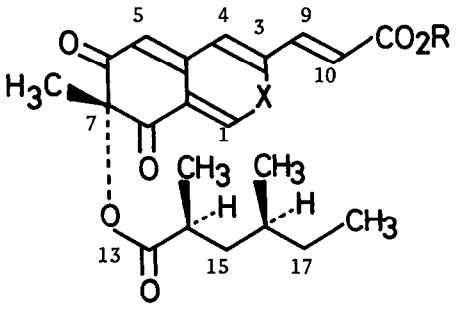
Mutual inhibition of the mycelial growth by the growing colonies of different strains of the same fungal species was first reported by Cayley in 1923[1], and this phenomenon was named aversion. It was several years before the discovery of the first antibiotic, penicillin.[2] Since then, aversion has been investigated in several kinds of fungi,[3] and active principles causing this phenomenon, which we called aversion factors, were previously assumed to be gaseous [3a] and volatile[3b] substances. Quite recently, we have shown that two strains of a phytopathogenic fungus, Cochliobolus setariae, caused aversion as the result of respective production by each strain of antibiotics, which inhibited selectively the growth of the opposite strain.[4] In continuing the research on this subject, half-aversion[1] was found among strains of a phytopathogenic fungus, Cochliobolus lunata. When ten strains[5] of this fungus were grown together on a agar medium, the IFO 5997 strain inhibited the growth of other eight strains leaving one strain (IFO 6586) unaffected, whereas the growth of IFO 5997 was not inhibited by any of the other nine strains tested. We wish now to describe isolation and structural elucidation of an aversion factor produced by the 5997 strain, naming it lunatoic acid A (1), and also of its closely related new metabolite, lunatoic acid B. We wish to emphasize that antibiotic reaction is found even among morphologically similar strains of a taxonomically identical fungal species.

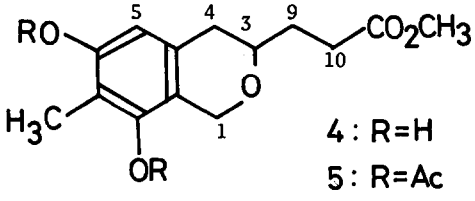
The 5997 strain was cultured in malt-dextrose medium by shaking for 3 days. Although free lunatoic acid A could be isolated from the ethyl acetate extracts of the culture filtrate by repeated tlc (Kieselgel 60pF$_{254}$; C_6H_6-HCOOEt-HCOOH), its purification was more easily performed by converting the extracts into the methyl ester with diazomethane. Thus, methyl ester of lunatoic acid A was purely obtained by subjecting the methylated extracts to successive column chromatography on silicic acid (CH_3OH in $CHCl_3$), Sephadex LH-20 (acetone), and silicic acid (ethyl acetate in benzene).

Lunatoic acid A methyl ester (2), yellow needles, had mp 109°, $[\alpha]_D^{26}$ -208° (c 0.17, $CHCl_3$), and $C_{22}H_{26}O_7$ (M^+:m/e 402.169; calcd. 402.168). Its UV spectra: λ_{max}(MeOH) 240 nm(ε 14100), 262 (15500), 348 (22000), 530 (1600); λ_{max}(MeOH-1N HCl) 240 nm(ε 15000), 270 (19000), 344 (19000); and λ_{max}(MeOH-1N NaOH) 253 nm(ε 17600), 355 (33600), 520 (6000) were indicative of presence of an amphipyrone chromophore in 2, because of their close similarity to the hypothetical UV deduced from that of mitorubrin.[6] The vinyl protons of 2 appeared in the nmr spectrum($CDCl_3$) at δ 7.92(bs, 1-H), 7.19(d, J=16 Hz, 9-H), 6.55(d, J=16 Hz, 10-H), 6.52(s, 4-H), 5.73(bs, 5-H), 1.56(s, 7-CH_3), and the ester methyl at δ 3.85, all of which agreed well with the proton signals reported on the amphipyrone chromophore of mitorubrinic acid.[7] The presence of this chromophore in 2 was supported by a characteristic reaction of 2 with ammonia, easily affording an expected

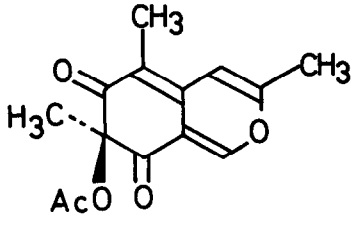
amine derivative (3), $C_{22}H_{27}O_6N$ (m/e 401.1860). 2 was hydrogenolyzed (Pd in MeOH) to an isochroman derivative (4), $C_{14}H_{18}O_5$ (m/e 266.112), λ_{max}(MeOH) 275 nm(ε 1200), 282 (1300). The product gave upon acetylation (Ac_2O, pyridine) the diacetate (5), $C_{18}H_{22}O_7$ (m/e 307.113; M^+-Ac), λ_{max}(MeOH) 264 nm(ε 500) and 272 (470), whose structure was completely ascertained by its nmr spectrum ($CDCl_3$), i.e., δ 6.79(s, 5-H), 4.65(ABq, J=15 Hz, 1-H), 4.23(m, 3-H), 3.70(s, $COOCH_3$), 2.70(d, J=6 Hz, 4-H), 2.52(t, J=7 Hz, 10-H), 2.29(s, two $COOCH_3$), ca. 2.0(m, 9-H), and 1.92(s, 7-CH_3). The acyl moiety ($C_8H_{15}O_2$) of 2 was clarified as follows. The pmr spectrum of 2 showed, besides the signals of the chromophore, a triplet methyl at δ 0.88(J=6 Hz), two doublet methyls at δ 0.91(J=6 Hz) and 1.19(J=7 Hz), the last signal was decoupled by irradiation of a multiplet signal at δ 2.65. These data strongly suggest the acyl part to be dimethylhexanoic acid. A fairly intense fragment ion $C_{17}H_{16}O_7$ (m/e 332.091), presumably arising from McLafferty rearrangement of the acyl moiety of 2, suggest that a secondary methyl group must be attached to the α position of the ester carbonyl. The three possible structures, i.e., 2,3-, 2,4-, and 2,5-dimethylhexanoic acid, were synthesized, and their methyl esters were compared in GC-Mass (5% OV-210, 3 mm χ 1 m, 50°C) with methyl ester of the acidic product obtained by alkaline hydrolysis of 2. Thus, the natural acid was found to be identical in the retention time (t_R=9.1 min) and mass spectrum with the 2,4-isomer, and its absolute stereochemistry was concluded from its optical rotation to be 2S, 4S, compared with its optically active compound synthesized by Odham.[8]



1 : R=H, X=O
2 : R=CH_3, X=O
3 : R=CH_3, X= NH
6 : R=CH_3, X=O , 9,10-dihydro-



4 : R=H
5 : R=Ac



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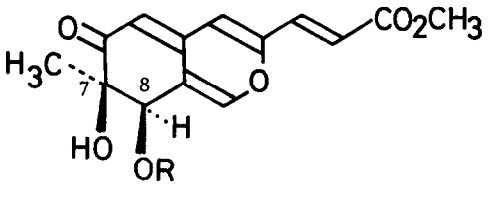
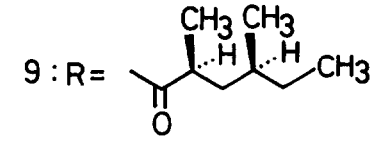
That the two moieties thus clarified are linked by an ester bond for building up the full structure was obtained from the mass spectra of 2 and 3, which showed respective intense fragment ions at m/e 276.063 ($C_{14}H_{12}O_6$; calcd. 276.063), 260.069 ($C_{14}H_{12}O_5$; calcd. 260.068) and at m/e 275.079 ($C_{14}H_{13}O_5N$; calcd. 275.079), 259.083 ($C_{14}H_{13}O_4N$; calcd. 259.082), all of which should be originated as the result of bond fission at the both sides of the ester oxygen. Cmr spectrum[9] confirmed the proposed structure of 2. The C-7 absolute stereochemistry of lunatoic acid A was

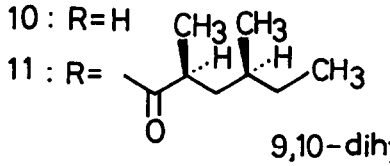
determined by CD of a dihydro-derivative (6), λ_{max}(EtOH) 220 nm(ε 16900), 329 (19000), which was prepared from 2 upon partial hydrogenation (Pd, ethyl acetate). Three Cotton effects at $\Delta\varepsilon_{351}$ -3.15, $\Delta\varepsilon_{274}$ +4.25, and $\Delta\varepsilon_{250}$ -0.48, agreed well, except the opposite sign, with those of the compound (7), whose absolute stereochemistry was unambiguously established to be S by an X-ray analysis of its bromo-derivative.[10] Thus, lunatoic acid A was shown to have the structure (1) with the 7R absolute stereochemistry.

Lunatoic acid B methyl ester (9), a yellow amorphous powder, showed $[\alpha]_D^{26}$ +201° (c 0.71, $CHCl_3$), $C_{22}H_{28}O_7$ (m/e 404.185; calcd. 404.184), λ_{max}(EtOH) 219 nm(ε 13500), 274 (12400), 283 (12300), 307 (10400), and 383 (11500). The compound had two more hydrogens than 2. These hydrogens were detected as a hydroxyl (δ 3.88; $\nu_{max}(CHCl_3)$ 3450 cm^{-1}) and a singlet methin (δ 5.62) protons, suggesting that either one of two carbonyl groups in the amphipyrone chromophore of 2 must be reduced to a secondary hydroxyl group. This assumption was confirmed by the cmr spectrum of 9, which showed only one ketonic carbon at δ 198.2(s), and a newly appeared oxygen-bearing methin carbon at δ 73.0(d). Evidence that the ketone reduced was at C-8 and not at C-5 was obtained from that (1) the C-1 vinyl proton in 9 appeared in the field considerably higher (Δδ 0.36 ppm) than the corresponding proton of 2, presumably owing to disappearance of an anisotropic effect by a ketonic function at peri-position, (2) on the other hand, the C-5 vinyl proton appeared in both compounds at similar chemical shift (Δδ 0.11 ppm). The hydroxyl function in 9 resisted against acetylation (Ac_2O, pyridine) and oxidation (CrO_3·2py.), and its proton signal appeared as a singlet in the pmr spectrum even in DMSO solution, suggesting that it must be tertiary. This indicates that the acyl moiety should be linked to the secondary, rather than tertiary, hydroxyl group. The acyl moiety of 9 was determined to be the same as (2S,4S)-dimethylhexanoate in 2 by the similar method as described above. A cis relation in the vicinal $C_{1,2}$-glycol in 9 was shown by the easy formation of an acetonide on the diol derivative (10), which was obtained from 9 by alkaline hydrolysis (K_2CO_3 in MeOH).

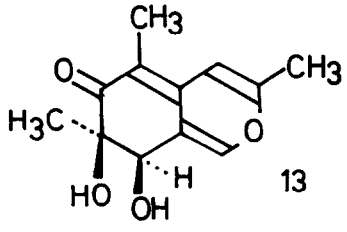




10 : R=H



9,10-dihydro-



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The absolute stereochemistry of 9 was determined by CD of a dihydro-derivative (11), λ_{max} (EtOH) 234 nm(ε 6000), 343 (19000), which was obtained from 9 by partial hydrogenation (Pd,

ethyl acetate). Three Cotton effects of 11, $\Delta\varepsilon_{352}$ +11.8, $\Delta\varepsilon_{310}$ -6.36, $\Delta\varepsilon_{235}$ -3.43, was well in accord with the values reported on 13, whose absolute stereochemistry was unambiguously determined as 7R,8R based on the X-ray analysis of its bromo-derivative.[10] Thus, lunatoic acid B was concluded to have the same absolute stereochemistry as that of 13. It is quite interesting to note that lunatoic acid A and B, possessing the opposite stereochemistry at C-7, were isolated from the same fungal culture.

Lunatoic acid A inhibited the growth of eight of nine strains of this fungus in the range of concentrations from 3 to 12 ppm. On the other hand, the growth of the IFO 6586 strain, which did not show aversion with the IFO 5997 strain, was inhibited by 100 ppm, and at this concentration the 5997 strain itself was also inhibited. Lunatoic acid A did not inhibit the growth of bacteria and fungi so far tested at 100 ppm, with only one exception, Pyricularia oryzae, a phytopathogenic fungus, that was inhibited at 50 ppm of lunatoic acid A.

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(9) $\delta(CDCl_3)$ 193.3(s, 6- or 8-C), 193.0(s, 6- or 8-C), 176.7(s, 13-C), 166.2(s, 11-C), 153.6(d, 1-C), 153.1(s, 3-C), 141.1(s, 4a-C), 134.0(d, 4- or 5-C), 124.3(d, 4- or 5-C), 116.2(d, 9-C), 115.3(s, 8a-C), 110.8(d, 10-C), 84.1(s, 7-C), 52.4(q, 21-C), 40.9(t, 15-C), 36.4(d, 14-C), 31.9 (d, 16-C), 29.6(t, 17-C), 22.0(q, 12-C), 19.1(q, 19-C), 17.7(q, 20-C), and 11.1(q, 18-C).

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