A NEW TOXIC FUNGAL METABOLITE, SILVATICAMIDE FROM ASPERGILLUS SILVATICUS

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Abstract --- In this brief report, the isolation and structure of silvaticamide which was isolated from a toxic strain of Aspergillus silvaticus as a toxic principle are described.

Aspergillus silvaticus(IFO 8173) was cultured on the sterilized rice(16.8Kg) stationary at 25°C for 4 weeks. The moldy rice was extracted with ethyl acetate and the extract residue(27.4g) was defatted with n-hexane. The obtained extract(16.3g) was chromatographed on a silica gel column with acetone-benzene and the toxic fractions were obtained. By an attempt to crystallization of the toxic fractions with acetone-benzene, a toxic metabolite was obtained as amorphous powder (1.02g). This metabolite seemed to be a new compound and designated as silvaticamide. The intraperitoneal administration of silvaticamide(208mg/Kg) to mice caused peritonitis and death within 4 days. The toxicity of the other chromatographic fractions(polar fractions) seemed to be due to the degradation products derived from silvaticamide but the isolation of the toxic components in those fractions was not carried out.

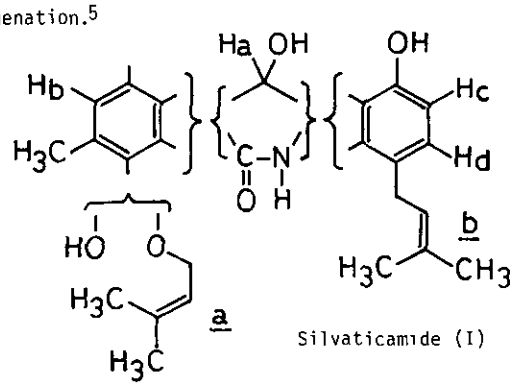
Silvaticamide (I), colorless amorph., mp 191-192°C(decomp.), $C_{25}H_{29}NO_5$, was optically inactive, and gave positive color-reaction(dark green) with $FeCl_3$, but negative with $Mg(OAc)_2$, Gibbs's, ninhydrin and Dragendorff's reagents. Silvaticamide was unstable to light and gradually decomposed to a mixture of purpish-red substances. In the ms spectrum, I afforded the ion-peaks at m/e(%) 423(M^+,20), 355(M^+-C_5H_8,100), 336(28), 282(18), 178(54), 123(19) and 68(20). The absorption maxima at nm(ε) 210(end absorp.,57500), 290(4400) and 310(5300) in methanol, and those at cm^{-1} 3390(OH), 3305(NH), 1660(amide C=O), 1598 and 1490(benzene ring) in KBr were observed in the uv and ir spectra, respectively. The molecular formula and the cmr and pmr data of I described below suggested that I is a tricyclic compound containing two benzene rings substituted with two isopentenyl groups. The cmr spectrum of I in CD_3OD showed that I consisted of five

methyls(δ, 16.3, 17.8, 18.1, 26.0(2C)ppm), two methylenes(28.9, 72.4ppm), one aliphatic methine (51.7ppm), five aromatic or olefinic methines(108,8, 122.1(2C), 123.9, 129.7ppm), eleven aromatic or olefinic carbons bearing no hydrogens(112.0, 121.2, 126.3, 133.1(2C), 134.3, 138.6, 148.8(2C), 155.0, 156.0ppm) and one carbonyl(172,8ppm). The pmr data in CD_3COCD_3 showed that one aromatic methyl(δ, 2.18(3H, s)ppm), and two isopentenyl groups, a(1.68(3H, s), 1.71(3H, s), 5.54(1H, broad (br.) t, $J_1=J_2=7$Hz), 4.69(2H, d, J=7)ppm) and b(1.66(3H, s), 1.67(3H, s), 5.23(1H, br. t, $J_1=J_2=7$), 3.19(2H, br. d, J=7)ppm) are present in I. The chemical shift of the methylene protons(4.69ppm) in a suggested that a is attached to an oxygen or a nitrogen atom. The signals of one methine proton Ha at 6.21(br. s), three aromatic protons Hb at 6.79(s), Hc at 6.41(d, J=8), Hd at 6.83(d, J=8) and four protons(disappeared with D_2O-addition) at 7.44(1H, br. s) and 7.45-8.19(3H, br. peak) ppm were also observed in the pmr spectrum. The chemical shift of Hc and Hd which were coupled each other(J=8Hz) suggested that Hc and Hd were located at ortho- and meta-positions to a phenolic hydroxyl group, respectively.[1,2,3,4] Further decoupling experiment showed that Hd was also coupled in long range(J<1) with the methylene protons in b(3.19ppm). Accordingly, a phenolic hydroxyl, Hc, Hd and one of two isopentenyl groups(possibly b) would be attached together to one benzene ring. Hb was found being coupled in long range(J<1) with the aromatic methyl(2.18ppm), indicating that Hb and the methyl group were located each other in ortho-position of the other benzene ring. It was shown that Ha was coupled(J<2) with the hydroxyl proton(7.44ppm), indicating that one secondary hydroxyl group is present in the molecule. The presence of the amide group was indicated in the ir spectrum. It is possibly expected that the amide group linked two benzene rings together with the carbon atom bearing the secondary hydroxyl group to make a tricyclic ring system and the remained functional groups, namely, one hydroxyl and another isopentenyl group(possibly a) would be attached also to the benzene ring joined with Hb and the aromatic methyl.(See Fig. 1).

On acetylation, I afforded triacetate(II), amorph., mp 137-138°C, $C_{31}H_{35}NO_8$(M^+: m/e549), λ_{max}^{MeOH} nm(ε) 276(sh.,2700) and 295.5(4600), ν_{max}^{KBr} cm^{-1} 3340, 1768 and 1702, negative to $FeCl_3$-test. The pmr spectrum of II in $CDCl_3$ indicated the presence of one aromatic and three acetyl methyls in the molecule. The broad peak observed at 7.45-8.19ppm in the spectrum of I disappeared, and the signal observed at 7.44ppm shifted to 6.04(br. s)ppm in the spectrum of II. II gave phenylurethane(III), amorph., mp 152-154°C, $C_{38}H_{40}N_2O_9$(M^+: m/e668). On further acetylation at 60°C for 1 day, II afforded tetraacetate(IV), amorph., mp 157-158.5°C, $C_{33}H_{37}NO_9$(M^+: m/e591), λ_{max}^{MeOH} nm(ε) 252(11100), 275(sh.,2800) and 310(4600), ν_{max}^{KBr} cm^{-1} 1770, 1732 and 1700, negative to $FeCl_3$. From the pmr data in $CDCl_3$, the presence of one aromatic and four acetyl methyls in IV was indicated. The fact that the signal which was observed at 5.41(br. s)ppm in the pmr spectrum of II shifted to 7.07(br. s)

ppm in the spectrum of IV suggested that the signal of the proton attached to the carbon bearing a secondary hydroxyl group shifted to the lower magnetic field by acetylation. The signal at 5.41 ppm in the pmr spectrum of II and that at 7.07ppm in the spectrum of IV were shown in a doubled form. The integral ratio of these signals was corresponding to a half of one proton. The signals at 5.58 and 7.12ppm in the spectra of II and IV were also observed in the same pattern. The reason of these facts will be discussed again later.

On catalytic hydrogenation with Pd-C in ethanol, IV afforded a product(V), amorph., mp 147-148°C, $C_{28}H_{31}NO_9$(M^+: m/e525), λ_{max}^{MeOH} nm(ε) 253(10700), 277(sh.,2100) and 321(6200), ν_{max}^{KBr} cm^{-1} 3370, 1765 and 1713, positive to $FeCl_3$. In the pmr spectrum in $CDCl_3$, the signals of one isopentyl group(0.87(3H, d, J=6) and 0.95(3H, d, J=6), 1.20-1.77(5H, m)) and of a proton at 8.55(s)ppm (disappeared with D_2O-addition) were newly observed, instead, the signals of the two isopentenyl groups observed in the spectrum of IV disappeared. These pmr data suggested that the isopentenyl group a in IV was eliminated by hydrogenation and a phenolic hydroxyl group was formed in V and the double bond in another isopentenyl group b was hydrogenated. It is well known that some phenyl allyl ethers are sometimes hydrogenolyzed to form phenols and olefines on catalytic hydrogenation.[5]



Silvaticamide (I)

Fig. 1

From the chemical and spectral data as described above, a partial structure of silvaticamide was assumed as shown in Fig. 1. Some fungal metabolites biogenetically regarded as so-called "seco-anthraquinones", namely, shamixanthone and tajixanthone[2] and variecoxanthone A, B and C[3] from Aspergillus variecolor, emericellin from A. nidulans[4] and arugosin A, B[1] and C[6] from A. rugulosus, have been reported to have the structures closely related to I. Especially, physico-chemical properties reported on arugosin B(VI)[1] were shown to be very close to those of I, except that I contains the nitrogen but VI does not. The comparison of the pmr spectra of I and VI compatibly indicated that the location of each functional group in both compounds is similar. When the position of the functional groups in I(shown in Fig. 1) was assumed to be the same to that shown in the structure of VI, the structure of silvaticamide should be proposed to be I as shown in Chart 1. The biosynthesis of I may be proceeded through the acetate-malonate pathway perhaps via the seco-anthraquinones as indicated in Chart 1. The structure of silvaticamide is thus deduced to be I on the basis of comparison of the spectral data with those of the biogenetically related fungal metabolites found in the literatures.[1,2,3,4,6]

Biogenesis of Silvaticamide

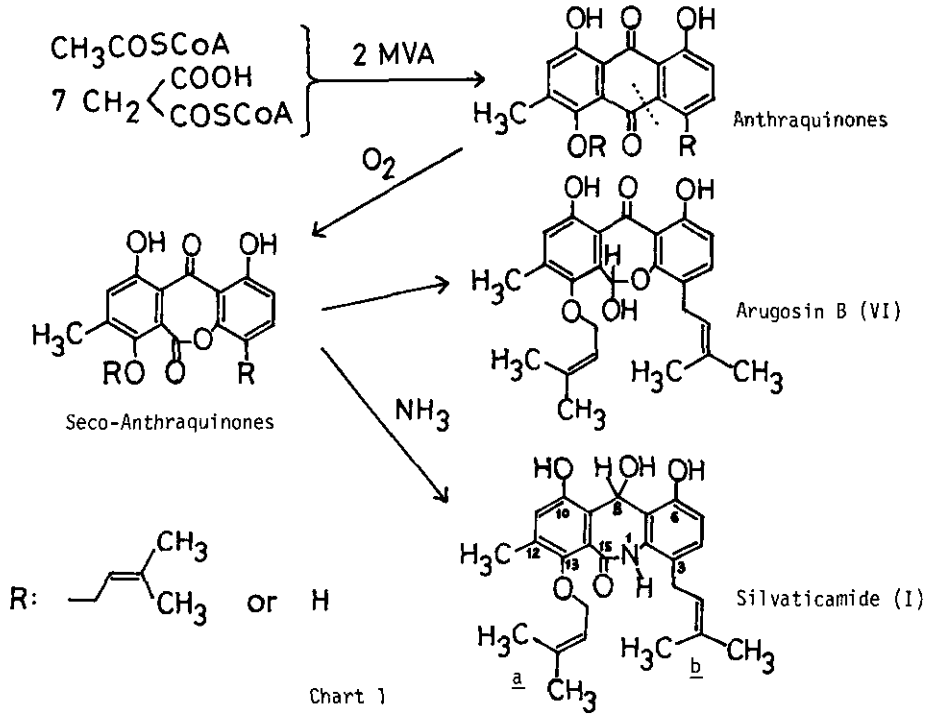


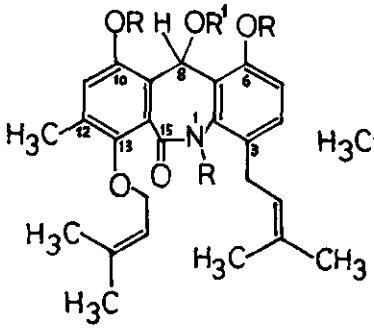
Chart 1

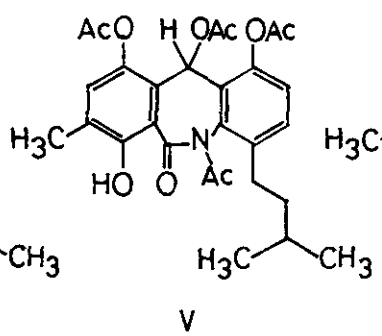
Controlled pyrolysis(180°C, 1-2mmHg, 1.5h) of II afforded two products, VII, prisms, mp 131-132°C, $C_{31}H_{35}NO_8$(M^+: m/e549), λ_{max}^{MeOH} nm(ε) 251(11900), 283(sh.,4800) and 310(4900), $\nu_{max}^{CCl_4}$ cm^{-1} 3310, 1774, 1740 and 1680, and VIII, fine prisms, mp 166-167.5°C, $C_{26}H_{27}NO_8$(M^+: m/e481), λ_{max}^{MeOH} nm(ε) 252(9600), 284(sh.,3500) and 321(5300), ν_{max}^{KBr} cm^{-1} 3385, 1763, 1707 and 1685(sh.), positive to $FeCl_3$. The uv spectrum of VII was rather different from that of II, but the molecular formula showed that VII is an isomer of II. The pmr spectrum of VII resembled to that of II. The pmr spectrum of VIII showed that VIII is a desisopentenyl derivative of VII. The structure of VII was determined by the direct x-ray diffraction analysis as shown in Chart 2, supporting that the location of the functional groups in I proposed above is correct.

I was shown to be optically inactive. In the pmr spectra of II and IV, some double signals of the acetyl methyls and the other protons were observed as mentioned above. These findings were assumed to be responsible to that I was isolated as a mixture of the stereoisomers regarding the configuration of the hydroxyl at C-8. The secondary hydroxyl at C-8 is rather labile due to its position at the benzylic carbon. The instability of I under light is possibly due to the quinonoid structure presumably formed by the photo-oxidation reaction initiating by the elimination of the isopentenyl group(a).

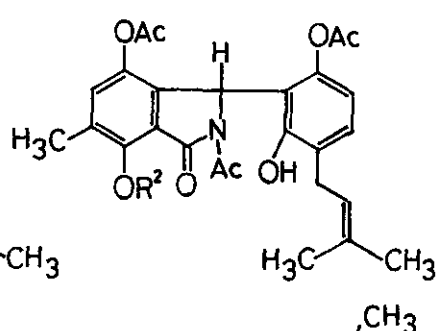
Recently, Homma et al.[7] have isolated an atrovenetin-like compound from A. silvaticus and determined the structure by x-ray analysis, however the biological activity of the compound

is not presented.





V

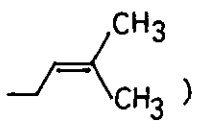


I (R : H, R^1 : H)

II (R : Ac, R^1 : H)

III (R : Ac, R^1 : $CONHC_6H_5$)

IV (R : Ac, R^1 : Ac)



VII (R^2 :)

VIII (R^2 : H)

Chart 2

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