

ISOLATION OF NEW ISOPRENYL ALCOHOLS FROM SILKWORM FECES

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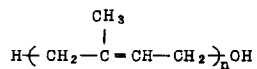
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New polyisoprenyl alcohols have been isolated from the unsaponifiable matter of silkworm (*Bombyx mori* L.) feces. Structural data on their substances are in agreement with structure I (n=11 and 12, which contain cis double bonds), undeca-isoprenol-1 and dodeca-isoprenol-1. From the above unsaponifiable matter, presence of solanesol(1-4)(n=9, all trans form) is now evident, also.



Structure I

First fractionation of the unsaponifiable matter obtained from lipid extract of silkworm feces was accomplished by molecular distillation. The fraction, distilled out from 200 to 230°C at  $10^{-3}$  Torr., was

then crystallized several times from n-hexane or acetone at  $-50^{\circ}\text{C}$  and was chromatographed over silica gel. Elution with 10 to 12% ether in n-hexane yielded two fractions as evidenced by thin layer chromatography and infrared spectrum. Each fraction gave only one spot on thin layer chromatography in a variety of solvent system.

The first fraction, which was a major component of the unsaponifiable matter, was obtained as colorless oil at room temperature.  $n_{\text{D}}^{25}$ : 1.5112,  $[\alpha]_{\text{D}}^{25} = 0$  and had no specific absorption in ultraviolet region. Its infrared spectrum showed primary OH absorption and unconjugated double bond,  $\nu_{\text{max}}^{\text{film}}$ : 3300, 3000, 1660, 1450, 1380, 1000, and  $830\text{ cm}^{-1}$ . Its nmr spectrum provided essential structural information (4~6),  $\tau$ (in  $\text{CDCl}_3$  reference with TMS, freq.  $60\frac{\text{Mc}}{\text{s}}$ )  
 $8.7\{-\text{OH}\}$ ,  $8.4\{-\overset{\text{CH}_3}{\text{C}}=\text{CH}(\text{trans})\}$ ,  $8.34\{-\overset{\text{CH}_3}{\text{C}}=\overset{\text{H}}{\text{C}}-(\text{cis})\}$ ,  $8.05\{-\text{CH}_2-\overset{\text{CH}_3}{\text{C}}=\}$ ,  
 $7.95\{-\text{CH}-\text{CH}_2-\}$ ,  $5.95(\text{d.})\{-\text{CH}-\text{CH}_2-\text{OH}, J=2.0\text{ cps}\}$  and  $4.80(\text{t.})\{-\overset{\text{CH}_3}{\text{C}}-\text{CH}-\text{CH}_2-\}$ .

Anal. found. C : 86.00%, H : 11.80%. The first fraction showed Rf 0.27 on silica gel G plates developed in benzene-chloroform(4:1), Rf 0.48 in benzene and Rf 0.84 in chloroform-methanol(4:1). From ozonization followed by reductive cleavage of the ozonide, levulin aldehyde and acetone were only found on gas chromatography and as 2, 4-dinitrophenyl hydrazones.

Hydrogenation of the first fraction yielded saturated isoprenyl alcohol derivative,  $n_{\text{D}}^{25}$ : 1.4630, Rf 0.22 in benzene-chloroform (4:1). The infrared spectrum of the hydrogenated product showed that in the reduction the absorption due to  $\nu_{\text{C}=\text{O}}$  had been shifted from 1000 to  $1050\text{ cm}^{-1}$  and its nmr spectrum was that also expected from a hydrogenated polyisoprenyl alcohol(5,7),  $\tau$ : 2.14(d.)  $[-\text{CH}_2-\overset{\text{CH}_3}{\text{C}}-]$ , 8.80  $[-\overset{\text{CH}_3}{\text{C}}-\text{CH}_2-\text{CH}-]$ , 6.45(t.)  $[\text{CH}_2-\text{CH}_2-\text{OH}]$ . But the hydroxyl proton signal was not obvious and it may have moved under the larger peaks.

However, results of reversed-phase paper chromatography showed

that the first fraction contains two substances. Further separation of the fraction was effected by the precise column rechromatography over silica gel. Spectral data and results of ozonization for the isolated substances ( $A_1$  and  $A_2$ ) were, however, indistinguishable from those of the described mother fraction except minor differences in relative area of proton in the nmr spectra. The area ratios between trans-methyl signal ( $\tau=8.4$ ) and cis-methyl signal ( $\tau=8.34$ ) in the nmr spectra were 1:2.0 for  $A_1$  and 1:1.7 for  $A_2$ . Above results showed that these two substances are both analogues of regular polyisoprenyl alcohol, which contain cis double bond in the molecules.

Isolated substance  $A_1$  showed Rf 0.43 on paraffin impregnated paper developed in N, N'-dimethyl formamide and Rf 0.33 in acetic acid,  $N_D^{25}$ : 1.5094, m.p. 9~10°C. A molecular weight determination by vapor pressure osmometer gave MW : 772, 756 (Undecaisoprenol 7673). Anal. Found. C : 86.11%, H : 11.93% Calcd. for  $C_{55}H_{90}O$  C : 86.09%, H : 11.82%. Quantitative catalytic hydrogenation indicated that it has 11.00 double bonds for structure I if  $n=11$ . This was carried out in a microhydrogenation apparatus at 20°C with Adams catalyst and ethanol-cyclohexane-acetic acid (1:1:1, by vol.) as solvent (5).

Substance  $A_2$  showed Rf 0.34 in N, N'-dimethyl formamide and Rf 0.28 in acetic acid,  $N_D^{25}$ : 1.5095, m.p. 15~16°C. MW : 837, 829 (Dodecaisoprenol 8354). Anal. Found. C : 86.19%, H : 12.00%, Calcd. for  $C_{60}H_{96}O$  C : 86.26%, H : 11.82%. Catalytic hydrogenation : 12.17 double bonds for structure I if  $n=12$ .

From our experimental results and a consideration of published works on solanesol (1~4), dolichol (5), spadicol (8) and betulaprenol (9), there can be no doubt that two substances we have isolated from the unsaponifiable matter of silkworm feces are undecaisoprenol-1 and dodecaisoprenol-1, respectively. Further work is in progress on confirmation of these structures.

The second fraction, Rf 0.17 on thin layer chromatography in benzene-chloroform (4:1), gave only single spot on reversed-phase paper chromatography in any solvent system (Rf 0.64 in acetone) and was obtained as white crystal from 98% acetone solution, m.p. 39~40°C. Its Rf values of thin layer and reversed-phase paper chromatography in any solvent systems agreed with those of authentic solanesol obtained from leaves of tobacco. In addition, spectral and analytical data of the crystal were quite identical with the data of solanesol. Therefore, the isoprenyl alcohol fraction II of silkworm feces was confirmed to be solanesol (all-trans nonaisoprenol-1).

These three isoprenyl alcohols were also found in the unsaponifiable matter of leaves of mulberry (*Morus bombycis* K.), which is the sole diet of silkworm, according to the procedure described above for those of silkworm feces. It is clear that these isoprenyl alcohols in silkworm feces originated from those in leaves of mulberry.

Detailed results will be published elsewhere.

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