with pMZ-D-Glu(γ-OBzl)-ONp (mp 125—126°, $[\alpha]_D$ +24°, Rf_1 0.92) to yield pMZ-D-Glu(γ-OBzl)-D-*a*Thr-D-Val-Leu-D-Ser-Leu-D-Ser-Ile-OBzl (V) (81%), mp 194—195°, $[\alpha]_D$ −16.5, Rf_1 0.32. After elimination of the pMZ-group by the method as described in IV, the resulting octapeptide was condensed with D-3-hydroxydecanoyl-Leu-$NHNH_2$[4] *via* the corresponding azide. The resulting protected N-acylnonapeptide D-Dec(OH)-Leu-D-Glu (γ-OBzl)-D-*a*Thr-D-Val-Leu-D-Ser-Leu-D-Ser-Ile-OBzl (VI) was isolated by column chromatography on silica gel using $CHCl_3$–MeOH–AcOH (95:5:3 v/v) as a solvent; 74%, mp 184—186°, $[\alpha]_D$ −18°, Rf_1 0.30. Finally the removal of the protecting group from VI by catalytic hydrogenation furnished D-Dec (OH) -Leu-D-Glu-D-*a*Thr-D-Val-Leu-D-Ser-Leu-D-Ser-Ile-OH (II) (73 %), mp 181—182°, $[\alpha]_D$ −10°; $[\alpha]_D$ −9.5° (c=1, EtOH) [*Anal.* Calcd. for $C_{54}H_{97}O_{17}N_9 \cdot 2H_2O$: C, 54.95; H, 8.64; N, 10.68. Found: C, 54.94; H, 8.57; N, 10.46. IR ν_{max}^{KBr} cm^{-1}: 1715 (C=O), 1630 (amide I), 1530 (amide II). Mass spectrum of the dimethyl ester *m/e*: 1171 (M^+). Amino acid ratios in acid hydrolysate (6N HCl, 110°, 24 hr): Glu 1.01, *a*Thr 0.89, Ser 1.78, Val 1.03, Ile 1.00, Leu 2.98], which gave a single spot (iodine-toluidine reaction) on TLC in different solvent systems (Rf_1 0.04, Rf_2 0.85, Rf_3 0.70) and was shown to be identical with viscosic acid obtained from the natural specimen in mp, mixed mp, $[\alpha]_D$, TLC, infrared (KBr) and mass spectra.

The dimethyl ester of the synthetic viscosic acid was prepared by CH_2N_2 treatment, which showed 203—205° and $[\alpha]_D$ −11°; the physical constants were identical with those of an authentic sample of viscosic acid dimethyl ester.

Confirmation of the structure of viscosic acid thus presents strong support to the assignd structure (I) of viscosin.

Acknowledgement The authors thank Dr. S. Yamaoka of Sumitomo Chemicals Co. supplying us with samples of viscosin.

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Received April 13, 1972

4) M. Hiramoto, K. Okada, S. Nagai, and H. Kawamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 1308 (1971).

(Chem. Pharm. Bull. 20(9)2065—2067(1972))

UDC 547.92'457.1.02 : 581.192

On the Tentative Structure of Wilforine

In 1966, Mitsuhashi, *et al.* reported the components of *Cynanchum Wilfordi* Hemsley (Japanese name Koikema), Asclepiadaceae family, containing polyoxypregnane ester glycosides.[1] They isolated three polyoxypregnane derivatives, sarcostin (I),[2] deacylmetaplexigenin (II),[3] and lineolon (III)[2] from the plants, after the alkali hydrolysis of the ester type aglycone mixture. In this communication, we wish to describe the separation and the structure of a new aglycone, wilforine (IV) from the ester type aglycone mixture.

1) H. Mitsuhashi, K. Sakurai, N. Kawahara, and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **14**, 712 (1966).
2) Y. Shimizu and H. Mitsuhashi, *Tetrahedron*, **24**, 4143 (1968); *idem*, *Chem. Pharm. Bull.* (Tokyo), **10**, 433 (1962).
3) H. Mitsuhashi and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **13**, 274 (1965).

The aglycone mixture, obtained after mild acid hydrolysis of the crude glycosides, was worked up by repeated silica gel column chromatography and preparative thin-layer chromatography (preparative TLC). These procedures yielded four crystalline compounds. Three of them were identified with penupogenin (V),[4] caudatin (VI),[5] and kidjolanin (VII)[6] by the comparison with authentic samples, respectively. The rest is a new aglycone, named wilforine (IV).

Wilforine (IV) shows the following properties; mp 158—163°, $[\alpha]_D$ +107° (c=1.05, chloroform), bluish violet with $SbCl_3$. *Anal.* Calcd. for $C_{37}H_{50}O_8$: C, 71.35; H, 8.09. Found: C, 71.25; H, 8.08. UV absorption λ_{max}^{EtOH} nm (log ε): 217 (4.43), 223 (4.39), 280 (4.33). IR absorption ν_{max}^{Nujol} cm^{-1}: 3550, 3380, 1708, 1643, 1580, 1500, 1180, NMR δ_{CDCl_3}: 0.99 (3H, d, J=6 Hz), 1.03, (3H, d, J=6 Hz), 1.15 (3H, s), 1.23 (3H, d, J=6 Hz), 1.53 (3H, s), 1.81 (3H, s), 3.50 (1H, m), 4.63 (1H, q, J=6 Hz), 4.76 (1H, d.d, J=4 and 12 Hz), 5.35 (1H, broad t, J=4 Hz), 5.69 (1H, s), 6.24 (1H, d, J=16 Hz), 7.38 (5H, m) 7.60 (1H, d, J=16 Hz). Hydrolysis of IV with 5% methanolic potassium hydroxide gave I and cinnamic acid. Gas chromatographic investigation of the mother liquor of the cinnamic acid showed the existence of ikemaic acid, VIII[7] (3,4-dimethyl 2-pentenoic acid).[8]

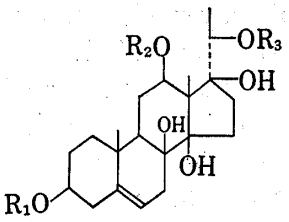
These facts suggest that wilforine (IV) is a diester of sarcostin (I) with cinnamic acid and ikemaic acid (VIII). The mass spectrum of IV showed no parent peak at m/e 622, however, other peaks agreed with this suggestion: m/e 604 ($M^+ - H_2O$), 586 ($M^+ - 2H_2O$), 474 (M^+ -cinnamic acid), 456 (474-H_2O), 436 (474-2H_2O), 346 (474-ikemaic acid), 328 (346-H_2O), 310 (346—2H_2O), 292 (346-3H_2O), 208 (346—138),[9a,b] 190 (208-H_2O), 131 (cinnamoyl cation), 111 (ikemaoyl cation).

Acetylation of IV with acetic anhydride in pyridine afforded a monoacetate (X), mp 126—132°, NMR δ 2.03 (3H, s, —OAc). The signal at δ 3.50 in the nuclear magnetic resonance (NMR) spectrum of IV shifted to 4.7 in that of X whose splitting pattern corresponded with that of 3α proton. Since both the signals at δ 4.63 and 4.76 in the NMR spectrum of IV are assigned to C-20 and C-12α protons, respectively, on the basis of each coupling pattern, these two ester linkages of IV exist on C-12β and C-20 positions in sarcostin (I). However it is not clear that either ikemaoyl or cinnamoyl group is attached to C-12β or C-20.

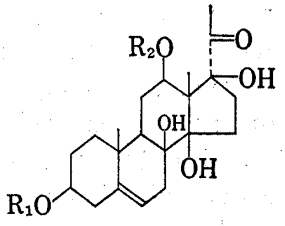
Reduction of IV with the equimolar amounts of $LiAlH_4$ in dioxane afforded I and IV. When the hydrolysis of IV under the above described conditions was stopped before the complete disappearance of IV, two kinds of partially hydrolyzed compounds were obtained in a very low yield. These two compounds have not cinnamoyl but ikemaoyl chromophore on the basis of the absorption maxima at 217 nm. One of them (12-O-ikemaoyl sarcostin) mp 160—170°, showed the peaks at m/e 492 (M^+), 474 ($M^+ - H_2O$), 456 ($M^+ - 2H_2O$), 447 ($M^+ - 45$), *etc.* on the mass spectrum. It is well known that the ion $M^+ - 45$ is originated from the cleavage of the glycol between C-17 and C-20 of pregnane derivatives[9b] indicating the existence of free hydroxyl group at C-20. Recently, Yamagishi, *et al.* found the phenomena that the acyl migration occurs reversibly between the 12β hydroxyl and the 20 hydroxyl groups on 17β-H C/D *cis* pregnane derivatives under alkaline or acidic condition.[10] Therefore, the other partially hydrolyzed product seems to be 20-O-ikemaoyl sarcostin.

4) H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **10**, 725 (1962).
5) T. Yamagishi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 625 (1972).
6) T. Sasaki, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 628 (1972).
7) 25% DEGS. 2 m glass column, column temp. 180°, carrier gas N_2, flow rate; 60 ml/min, t_R 8.4 min.
8) H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **8**, 318 (1960).
9) retro-Diels-Alder cleavage of Δ^5 in B-ring; *a*) B. Kapur, H. Allgeier, and T. Reichstein, *Helv. Chim. Acta*, **50**, 2147 (1967); *b*) M. Fukuoka, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1469 (1971).
10) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, The 92nd Annual Meeting of the Pharmaceutical Society of Japan, Abstract Papers, Ohsaka, II, 1972, p. 174.

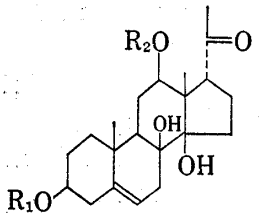
From these results, it is deduced that descinnamoylwilforine (one of the above ikemaoylsarcostins) is formed first from (IV) and the subsequent migration of the ikemaoyl group causes the formation of another ikemaoylsarcostin under the alkaline conditions.



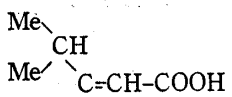
I: $R_1=R_2=R_3=H$
IV: $R_1=H$, $R_2=$cin, $R_3=$ikem
V: $R_1=R_3=H$, $R_2=$cin
X: $R_1=$Ac, $R_2=$cin, $R_3=$ikem
XII: $R_1=$Ac, $R_2=$ikem, $R_3=H$
XIII: $R_1=$Ac, $R_2=$ikem, $R_3=$cin
cin: cinnamoyl
ikem: ikemaoyl



II: $R_1=R_2=H$
VI: $R_1=H$, $R_2=$ikem
VII: $R_1=H$, $R_2=$cin
XI: $R_1=$Ac, $R_2=$ikem



III: $R_1=R_2=H$
IX: $R_1=H$, $R_2=$ikem



VIII

In order to confirm the position of the ester linkages of IV the following experiments were carried out. Caudatin acetate (XI)[5] was reduced with $NaBH_4$. The product (XII) mp 193—199°, δ 1.16 (3H, d, J=6 Hz, C-21-Me), 3.62 (1H, q, J=6 Hz, C-20-H), was treated with cinnamoyl chloride in pyridine to give 3-O-acetyl, 12-O-ikemaoyl, 20-O-cinnamoyl sarcostin (XIII) which was not identified with wilforine acetate (X) on TLC. The finding suggests that the structure of wilforine is given in terms of the formula (IV).

Acknowledgement We wish to express our thanks to Professor Dug-Ryong Hahn (Seoul) for collection of the plants. Thanks are due to Mrs. Araki for 100 MHz spectral measurement, to Miss Kakizaki for elemental analysis, and to Miss Takahashi for mass spectral measurenent.

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Received April 24, 1972

(Chem. Pharm. Bull. 20(9)2067—2069(1972))

UDC 547.789.1.04 : 547.571.04

Chemistry of 2-Substituted Thiothiazoline. V. Reaction of Dianion of 2-Propargylthiothiazoline with Benzaldehyde

In the preceding communication we reported the unique reactivities of the dianion of proparglythiothiazoline and the 1,5-diyne system synthesis using these 2-alkynylthiothiazolinelithium species.[1] It is of interest to investigate further the properties of these species in the reactions with other electrophiles such as aldehyde. In this viewpoint, we studied the reactions of 2-proparglythiothiazolinelithium and 2-phenylproparglythiothiazolinelithium with benzaldehyde.

1) K. Hirai and Y. Kishida, *Tetrahedron Letters*, **1972**, 2117.