of water), the production of fumitremorgins was almost of non-detectable. However, the addition of L-tryptophan to the medium caused abundant production of the toxins as shown in Table I.

Substantially, the efficient incorporation of the radioactivity to fumitremorgin A and B from DL-tryptophan-3-^{14}C has been confirmed in a tracer experiment, and the result strongly supports that fumitremorgin A and B contain the indole ring in those structures as assumed above. As the metabolites of *A. fumigatus* Fres., some indolic compounds have been isolated, such as agroclavine, erymoclavine, festuclavine, chanoclavine, fumigaclavine[3] or sulphur containing gliotoxin.[4] However, fumitremorgin A and B are obviously differed in their spectral and chemical properties from the above metabolites.

The intraperitoneal injection of 1 mg of fumitremorgin A and B causes sustained trembling with intermittent convulsion to mouse. The tremor action on mice normally appears five minutes after injection and continued for several hours. The injection of fumitremorgin B causes usually more severe convulsion than that by A. No lethal examples are observed in a dose of 1 mg of the pure toxins, however the death of 70% of animals is observed within 96 hours by the administration of 5 mg.

The investigation on the chemical structure of these compounds is still going on and details of the study will be reported elsewhere in near future.

Institute of Food Microbiology,
Chiba University
3-9-1, Izumi-cho, Narashino,
Chiba

MIKIO YAMAZAKI
SHIGETOSHI SUZUKI
KOMEI MIYAKI

Received May 1, 1971

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[Chem. Pharm. Bull. 19(8)1740—1742(1971)]

UDC 547.597.02 : 581.192

On the Structure of Senegin-II of Senegae Radix

As we reported in the previous paper,[1] four glycosides, namely senegin-I, -II, -III and -IV, were isolated from the *n*-BuOH soluble fraction of MeOH extract of Senegae Radix (root of *Polygala senega* LINNE var. *latifolia* TORRY et GRAY (Polygalaceae)).

Senegin-II (I), $C_{70}H_{104}O_{32}\cdot 4H_2O$, mp 247—248°, colorless needles from *n*-BuOH–AcOH–H_2O (4:1:5, upper layer), $[\alpha]_D^{20}$—6.2° (c=2.0, MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3500—3300 (OH), 1750 1730 (COOR), 1710 (COOH), 1635 (C=C), 1610, 1515 (benzenoid), UV λ_{max}^{EtOH} mμ (log ε) 317 (4.28), is composed of presenegenin, 3,4-dimethoxycinnamic acid, glucose, galactose, rhamnose, fucose and xylose.

On methylation with CH_2N_2 in MeOH, I gave a monomethyl ester (II), which was acetylated with acetic anhydride and pyridine to afford senegin-II monomethyl ester tetradecaacetate (III), $C_{99}H_{134}O_{46}\cdot 2H_2O$, colorless powder, mp 164—166°. Acid hydrolysis of II

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with 0.03N H_2SO_4-dioxane yielded five products (IV, V, VI, VII, VIII). The first product, IV, $C_{37}H_{58}O_{12}\cdot 2H_2O$, colorless needles from AcOEt saturated with water, mp 231—232°, IR ν_{max}^{Nujol} cm^{-1}: 3400—3500, 1725, 1690, 1630, NMR $\delta_{TMS}^{CDCl_3}$: 3.8 ($COOCH_3$), was acetylated with acetic anhydride and pyridine to give a pentaacetate (IX), $C_{47}H_{68}O_{17}\cdot H_2O$, colorless needles from MeOH, mp 215—217°, IR ν_{max}^{Nujol}: 3560, 1740 (with inflection at 1760), 1725, 1250, NMR $\delta_{TMS}^{CDCl_3}$: 1.97 (3H(s)×3, $OCOCH_3$), 2.0 (3H(s), $OCOCH_3$), 2.01 (3H(s), $OCOCH_3$), 3.7 (3H(s), $COOCH_3$), 5.53 (1H(q), -C=CH). The pentaacetate (IX) was methylated with CH_2N_2 to give a methyl ester(X), $C_{48}H_{70}O_{17}$, colorless needles from $CHCl_3$–hexane, mp 220—222°, IR ν_{max}^{Nujol} cm^{-1}: 3580, 1760, 1740 (broad), NMR $\delta_{TMS}^{CHCl_3}$: 2.00 (3H(s)×3, $OCOCH_3$), 2.01 (3H(s), $OCOCH_3$), 2.02 (3H(s), $OCOCH_3$), 3.63 (3H(s), $COOCH_3$), 3.70 (3H(s), $COOCH_3$), 5.55 (1H (q), >C=CH).

The properties of X suggest that it must be presenegenin 3-O-β-D-glucoside dimethyl ester pentaacetate which was reported by S.W. Pelletier,[2] and X was identified with the authentic sample by mixed fusion and comparison of infrerad (IR) spectra.

IX was treated with bromine to give monobromolactone (XI),[3] $C_{47}H_{67}O_{17}Br$, 121—123°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780 (lactone). Therefore, the methyl ester group is located at C-4 of presenegenin.

The second product, V, $C_{11}H_{20}O_{10}$, colorless cubes from EtOH, mp 173°, was hydrolysed with dilute acid to give xylose and galactose. The results of degradation reaction and the physical constant suggest V to be 4-β-D-galactopyranosyl-D-xylose.[4]

The hydrolysates of VI and VII were examined by thin-layer chromatography (TLC) and gas–liquid chromatography (GLC) to reveal the presence of glucose, fucose, 3,4-dimethoxycinnamic acid and presenegenin in the hydrolysate of VI and glucose, fucose, rhamnose, 3,4-dimethoxycinnamic acid and presenegenin in that of VII, respectively.

The product (VIII) gave fucose and 3,4-dimethoxycinnamic acid on acid hydrolysis.

The foregoing experimental data suggest that (a) one of the carboxyl groups of I is present in free form at C-4 and the other at C-17 in ester form. (b) 3,4-Dimethoxycinnamic acid of I is attached to fucose.

On treatment with excess of CH_2N_2 in MeOH, I gave des-3,4-dimethoxycinnamoyl senegin-II monomethyl ester (XII). Acetylation of the latter (XII) afforded a pentaacetate (XIII), $C_{90}H_{126}O_{44}$, colorless needles from EtOH, mp 168—170°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 1750, 1630, 1240, NMR $\delta_{TMS}^{CDCl_3}$: 3.8 (3H(s), $COOCH_3$), 2.0—2.3 (3H(s)×15, $OCOCH_3$). Permethylation of I and XII by Hakomori's method[5] gave per-O-methylsenegin-II (XIV), $C_{85}H_{134}O_{32}$, colorless powder from hexane, mp 140—142°, and des-3,4-dimethoxycinnamoyl-senegin-II monomethyl ester pentadeca-O-methylether (XV), $C_{75}H_{126}O_{29}$, colorless powder from hexane, mp 125—127°, respectively.

On reduction with $LiAlH_4$ in tetrahydrofuran, XIV gave XVI, $C_{41}H_{68}O_{10}$, colorless powder, mp 125—126° from the ether extract of the reaction mixture and XVII, $C_{32}H_{60}O_{18}\cdot 1/2H_2O$, colorless powder, mp 59—60° from the $CHCl_3$ extract. The partially O-methylated sugars of each hydrolysate of XVI and XVII were examined by TLC and GLC to show the presence of per-O-methyl-D-glucose in the former, and per-O-methyl-galactose, 2,3-di-O-methyl-rhamnose, 2,3-di-O-methylxylose and partially O-methylated fucitol in the latter.

The hydrolysate of XIV contains 3-O-methyl-fucose, 2,3-di-O-methylrhamnose, 2,3-di-O-methyl-xylose, 2,3,4,6-tetra-O-methyl-galactose and 2,3,4,6-tetra-O-methyl-glucose, while that of XV includes 3,4-di-O-methyl-fucose, 2,3-di-O-methyl-rhamnose, 2,3-di-O-methyl-xylose, 2,3-4,6-tetra-O-methyl-galactose and 2,3,4,6-tetra-O-methyl-glucose. By the analysis

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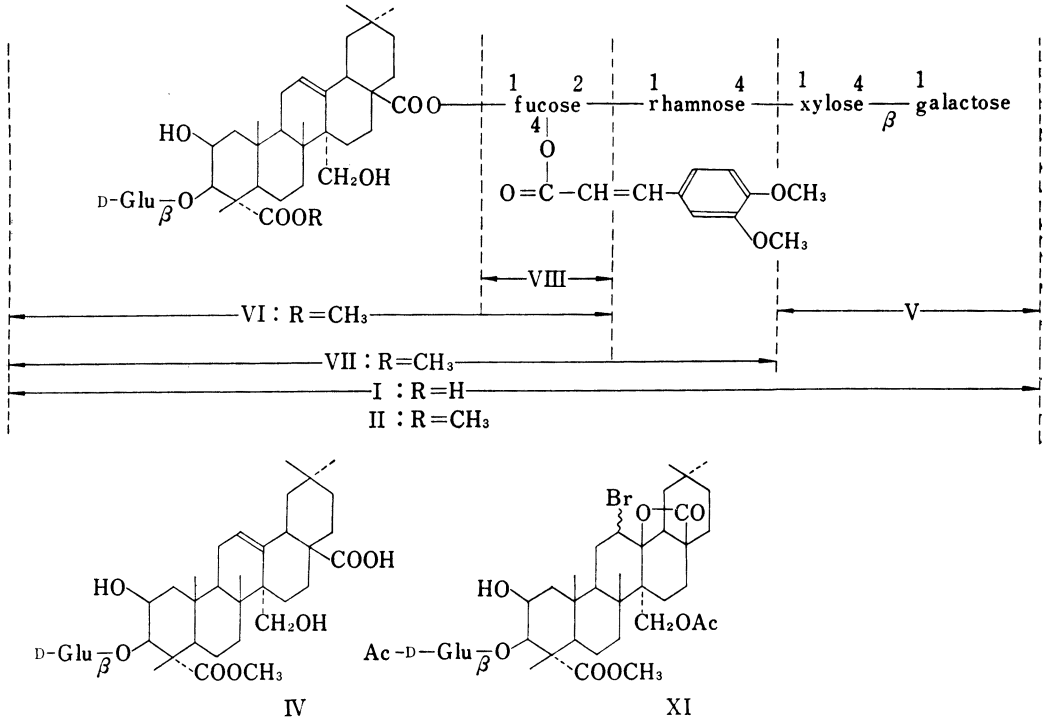

Fig. 1

of these O-methylated sugars, 3,4-dimethoxycinnamic acid moiety of I is proved to be present at C-4 hydroxyl group of fucose.

Thus the structure of senegin-II is formulated as I. The nuclear magnetic resonance spectra of IV and V revealed that glucose and galactose in the saponin are linked with β configuration. The configurations of other sugars are now under investigation.

The study on the structures of senegin-III and IV will be published in the near future.

Acknowledgement The authors express their gratitude to Prof. T. Kawasaki, University of Kyushu, Prof. S.W. Pelletier, The University of Georgia, Dr. E.E. Percival, University of London, and Associate Prof. E.G. Gros, Universidad De Buenos Aires for their kind supply of the authentic samples. Thanks are also due to Mr. H. Ishizone for his co-operation in this work, to the members of Analytical Laboratory of Showa University for elemental analysis.

School of Pharmaceutical Sciences,
Showa University
Shinagawa-ku, Tokyo

JUNZO SHOJI
SACHIKO KAWANISHI

Iyakushigen Institute for Medical Research
Koganei-shi, Tokyo

YASUMASA TSUKITANI

Received March 30, 1971