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82. **Akira Yagi and Toshio Kawasaki**: Structure of a New Ester-Alkaloid from *Veratrum grandiflorum* (MAXIM.) LOESENER *fil.*
(Alkaloids of Japanese Veratrum Genus Plants. IV.[*1])

(*Institute of Pharmaceutical Sciences, Faculty of Medicine, Kyushu University*[*2])

In the preceding paper[*1] of this series it was reported that a new ester-alkaloid (Ea) was isolated from the rhizomes of *Veratrum grandiflorum* (MAXIM.) LOESENER *fil.* and found to be easily hydrolyzed with 5% sodium hydrogen carbonate-methanol to give one mole each of angelic acid and the alkamine which had been obtained[1a] from *Veratrum oxysepalum* TURCZ. and characterized[1b] by Shimizu as zygadenilic acid δ-lactone[*3] (A) (Ia).

This paper deals with a study on the structure of the (Ea) in which the angelic acid residue is supposed to be attached to one of the secondary hydroxyl groups, at C_{15} or C_{16}, of the alkamine (A).

(Ea) (Ib) when treated with acetone and hydroiodic acid gave a monoacetonide (Ⅱb). Since (A) has only one *cis* α-glycol grouping at C_{14} and C_{15} to form the monoacetonide[2] (Ⅱa), (Ⅱb) should also be a 14,15-acetonide, and this suggests the presence of the free hydroxyl groups at the corresponding positions in (Ea).

When treated in the same manner as (A) was acetylated to (A)-acetate (Ⅲa), (Ea) (Ib) provided (Ea)-monoacetate (Ⅲb). Because (Ⅲa) is regarded as a 15,16-diacetate[1,3] and, as mentioned above, the hydroxyl group at C_{15} in (Ea) should be the free state, it is most likely that (Ⅲb) is the 15-acetate 16-angelate of (A). Furthermore, while both (A)-acetonide and (Ea)-acetonide suffered no oxidation in dioxane by 0.1*N* periodic acid at 24°C for 3 hours and were recovered unchanged, (A) and (Ea) consumed under the above condition 1.8 and 0.8 mole periodic acid, respectively, and yielded the oxidation products both of which showed the infrared absorption band at 1740 cm^{-1} (five-membered ring ketone). It indicates that in (Ea) either one at C_{14} or C_{16} out of the three vicinal hydroxyl groups participated in the angelate formation. Since the ester-linkage at the 14-hydroxyl group is ruled out from the afore-mentioned data, it is concluded that (Ea) is 16-angelate of (A) (Ia).

No natural veratrum alkaloid occurring as a 16-ester has been reported except for isogermidine (germine 15-(*l*)-2-methylbutyrate 16-acetate[4]) and (Ea) is the second one of this kind.

In the cevine and germine analogs which are considered to possess 16-β, 20-β-(1,3-diaxal) hydrolyl groups,[5] it is known that 16-acetates of their derivatives undergo ready methanolysis[5,6] and lability of the ester-linkage is increased by the hydroxyl group at

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[*1] This paper constitutes part of the series "Takeo Tsukamoto: Alkaloids of Japanese Veratrum Genus Plants." Part Ⅲ. T. Tsukamoto and A. Yagi: Yakugaku Zasshi, **79**, 1102 (1959).

[*2] Katakasu, Fukuoka (八木 晟, 川崎敏男).

[*3] The term "zygadenine δ-lactone" which appeared in Part Ⅲ[*1] of this series should be corrected as zygadenilic acid δ-lactone.[1b]

1) *a*) B. Shimizu, M. Suzuki: Yakugaku Zasshi, **79**, 609 (1959). *b*) B. Shimizu: *Ibid.*, **79**, 993 (1959).
2) S.M. Kupchan: J. Am. Chem. Soc., **81**, 1925 (1959).
3) S.M. Kupchan, D. Lavie, R.D. Zonis: *Ibid.*, **77**, 689 (1955).
4) S.M. Kupchan, C.R. Narayanan: Chem. & Ind. (London), **1956**, 1092.
5) *a*) Cevine: D.H.R. Barton, *et al.*: J. Chem. Soc., **1954**, 3950. S.M. Kupchan, *et al.*: J. Am. Chem. Soc., **78**, 3864 (1956); *Ibid.*, **80**, 1769 (1958); Tetrahedron, **7**, 47 (1959). *b*) Germine: S.M. Kupchan: J. Am. Chem. Soc., **81**, 1921 (1959). *c*) Protoverine (6α-hydroxygermine): *Idem*: *Ibid.*, **82**, 2242 (1960). *d*) Zygadenine (7-desoxygermine): *Idem*: *Ibid.*, **81**, 1925 (1959).
6) W.J. Rosenfelder: J. Chem. Soc., **1954**, 2638.

C_{20}.[5,7] Recently it has also been reported[8] that synthetic 4,16-diisobutyrate of a protoverine derivative suffers methanolysis, though the rate is much decreased. Since (A) was shown by Shimizu[1b] to have the substituents of the same configurations at C_{14}, C_{15}, C_{16}, and C_{20} as zygadenine (7-desoxygermine[2]), the angelic acid residue at 16-hydroxyl group of (Ea) and (Ea)-acetonide was also expected to be removed on treatment with methanol and this was actually found to be the case.

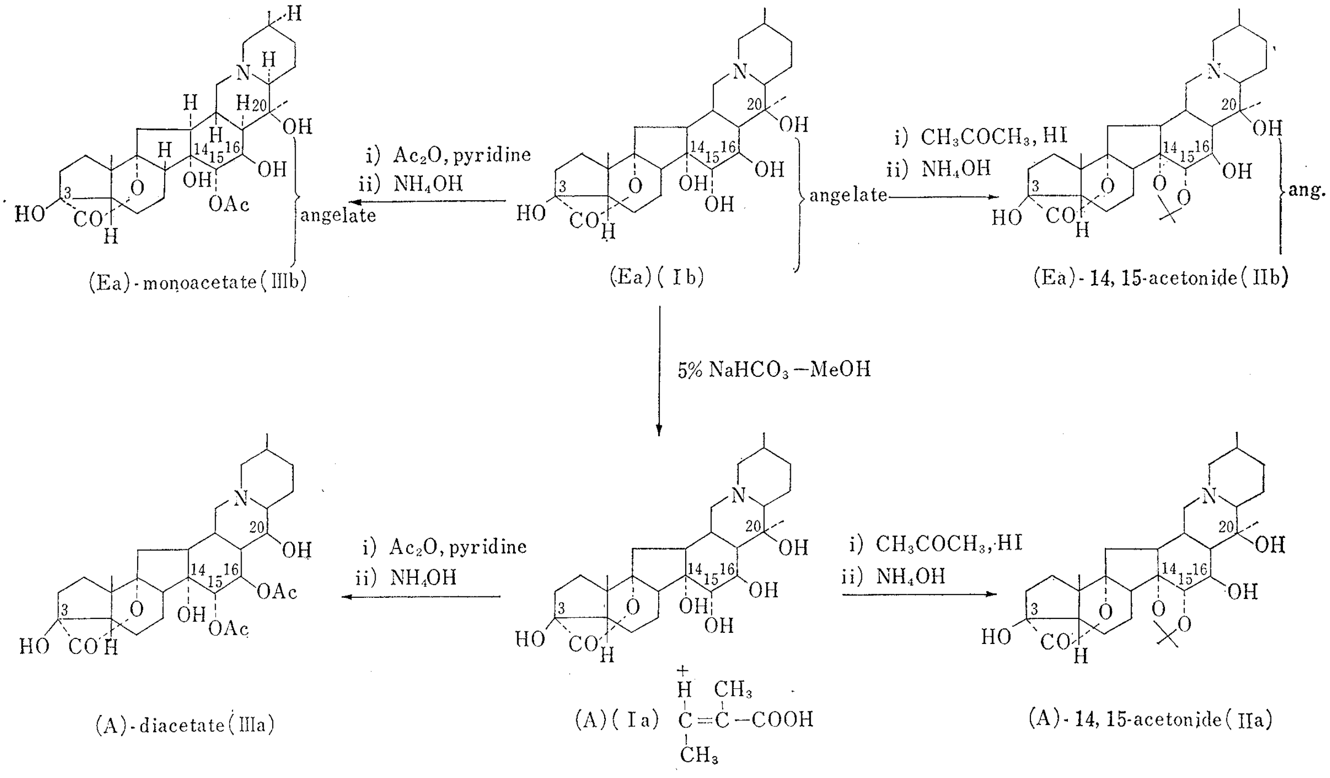

Chart 1.

Experimental*4

Zygadenilic Acid δ-Lactone Angelate Monoacetonide (**(Ea)-monoacetonide**) (**IIb**)——A solution (pH 4.0) of (Ea) (Ib) ($C_{32}H_{47}O_8N \cdot H_2O$)*1 (500 mg) m.p. 235 (decomp.), $[\alpha]_D^{15}$ −10.9°(c=1.65), and 57% HI (0.5 cc.) in Me_2CO (2 cc.) was kept standing for 1 hr. at room temperature. Crystals separated out were collected by filtration and recrystallized from MeOH–Me_2CO to give 510 mg. of monoacetonide-HI as needles, m.p. 271°(decomp.). *Anal.* Calcd. for $C_{35}H_{51}O_8N \cdot HI \cdot 2H_2O$ ((Ea)-monoacetonide $HI \cdot 2H_2O$): C, 54.05; H, 7.26; N, 1.80. Found: C, 54.46; H, 7.55; N, 1.93. A solution of the hydroiodide (500 mg.) in H_2O was ice-cooled, adjusted to pH 9.5 with dil. NH_4OH and extracted with $CHCl_3$. $CHCl_3$ layer was washed with H_2O, dried and evaporated to dryness. The residue was chromatographed on Al_2O_3 (washed with dil. H_2SO_4, slightly acidic) and the eluate with benzene gave 350 mg. of (IIb), m.p. 206° (from Me_2CO), $[\alpha]_D^{33}$ −43°(c=0.12). *Anal.* Calcd. for $C_{35}H_{51}O_8N \cdot H_2O$ ((Ea)-monoacetonide·H_2O): C, 66.54; H, 8.46; N, 2.22. Found: C, 66.47; H, 8.81; N, 2.21. IR ν cm^{-1}: 1724 (ester C=O), 1653 (C=C).

Zygadenilic Acid δ-Lactone Monoacetonide (**(A)-monoacetonide**) (**IIa**)——(A) (Ia) (500 mg.) was treated in the same manner as described above to afford 360 mg. of (IIa), m.p. 281° (from benzene), $[\alpha]_D^{33}$ −43.3°(c=0.16). *Anal.* Calcd. for $C_{30}H_{45}O_7N$ ((A)-monoacetonide): C, 67.77; H, 8.53; N, 2.63. Found: C, 67.65; H, 8.66; N, 2.73. IR ν cm^{-1}: 1733 (δ-lactone C=O), 1080 (alcohol C–O).

Zygadenilic Acid δ-Lactone Angelate Monoacetate (**(Ea)-monoacetate**) (**IIIb**)—(Ea) (Ib) (137 mg.) was kept standing with Ac_2O (2 cc.) and pyridine (0.8 cc.) at 24° for 16 hr. and Ac_2O and pyridine were removed

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*4 All melting points were taken on a Kofler block and are uncorrected. The infrared spectra were determined in Nujol mulls, and optical rotations measured in dioxane.

7) H.B. Henbest, B.J. Lovell: *Ibid.*, **1957**, 1965.

8) S.M. Kupchan, C.I. Ayres: J. Am. Chem. Soc., **82**, 2252 (1960).

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in vacuo. To the residue dil. NH_4OH was added under cooling, the mixture adjusted to pH 9.5 and extracted with $CHCl_3$. $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4 and evaporated. The residue was dissolved in benzene, and passed through a column of 15 g. of silica gel-celite (3:1) (holding 10 cc. of 0.2*M* acetate buffer of pH 3.6, as a stationary phase). Benzene eluate afforded 110 mg. of (IIIb), m.p. 182°(from Me_2CO-petr. ether), $[\alpha]_D^{33}$ −32°(c=0.15). *Anal.* Calcd. for $C_{34}H_{49}O_9N \cdot 2H_2O$ ((Ea)-monoacetate·$2H_2O$): C, 62.64; H, 8.19. Found: C, 62.68; H, 7.70. IR ν cm^{-1}: 1750 (C=O), 1724 (ester), 1653 (C=C), 1240 (ester C–O).

Zygadenilic Acid δ-Lactone Diacetate ((A)-**diacetate**) **(IIIa)**——(A) (Ia) (400 mg.) was acetylated and worked up in the same way as in (Ib). The crude acetate was chromatographed on Al_2O_3 and the eluate with petr. benzin furnished 120 mg. of (IIIa) as needles (from petr. benzin), m.p. 250~253°. *Anal.* Calcd. for $C_{31}H_{45}O_9N$ ((A)-diacetate): C, 64.67; H, 7.88. Found: C, 64.64; H, 8.29.

Oxidation of (A)-acetonide (IIa), (Ea)-acetonide (IIb), (A) (Ia) and (Ea) (Ib) by Periodic Acid——Sample (30 mg.) in dioxane (5 cc.), H_2O (10 cc.) and 0.1*N* HIO_4(5.00 cc.) were allowed to stand at 24° for a given time. The reaction mixture was made alkaline with dil. $NaHCO_3$ solution, 0.1*N* As_2O_3 solution was added, and the mixture was titrated with 0.1*N* I_2 solution. The titrated solution was extracted with $CHCl_3$, $CHCl_3$ layer washed with H_2O, dried and evaporated to dryness. The residue was examined by infrared analysis. The results are shown in Table I.

Table I. Periodic Acid Oxidation

Substrate	Molar equivalents of HIO_4 consumed (reaction time, hr.)	Product
(A)-acetonide (IIa)	0 (2), 0 (3), 0 (24)	Recovered (IIa)
(Ea)-acetonide (IIb)	0 (2), 0 (3)	〃 (IIb)
(A) (Ia)	1.5(2), 1.8(3)	Five-membered ring ketone ($\nu_{C=O}$ 1740 cm^{-1})
(Ea) (Ib)	0.8(2), 0.8(3)	〃

Methanolysis of (Ea) and (Ea)-acetonide——A sample solution (20 mg. of (Ea) or (Ea)-acetonide in 50% MeOH (2 cc.)) was refluxed on a water bath for a given time, evaporated *in vacuo* and the residue was examined by paper chromatography according to the method reported.[9] The results are ahown in Table II.

Table II. Methanolysis with 50% Methanol and Paper Chromatographic Examination[9] of their Products

Sample	(Ea) (Ib)					(Ea)-acetonide (IIb)					
Refluxing time (hr.)	2	8	11	24	52	2	8	11	24	52	72
Product: 16-Angelate unchanged Rf (Ea) 0.02 (Ea)-acetonide 0.10	++	++	+	+	+	++	++	++	+	+	+
Product: 16-OH-Compd. Rf (A) 0.00 (A)-acetonide 0.01	−	−	+	+	++	−	−	−	+	+	++

Solvent system: benzene-PrOH-HCOOH (15:3:1) Temp.: 24°; Time: 3 hr.
Rf values of reference compounds run in parallel.
(Ea) (Ib): 0.02 (Ea)-acetonide (IIb): 0.11
(A) (Ia): 0.00 (A)-acetonide (IIa): 0.02

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Summary

A new ester-alkaloid isolated from the rhizomes of *Veratrum grandiflorum* (Maxim.) Loesener *fil.*, m.p. 235°, $[\alpha]_D^{15}$ −10.9°, was found to be 16-angelate of the alkamine which had been obtained from *Veratrum oxysepalum* Turcz. and characterized by Shimizu as zygadenilic acid δ-lactone.

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[9] A. Yagi, T. Kawasaki: Yakugaku Zasshi, 82, 300 (1962).

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