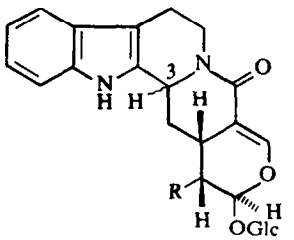
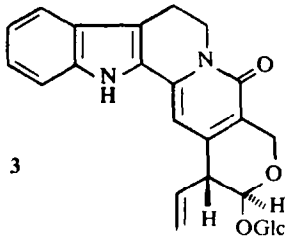
purified by a simple counter-current distribution. After three transfers an organic concentrate of indolic material was obtained, which was washed with pH 9 buffer to remove the phenolic cinnamates. Removal of the solvent gave an amorphous yellow powder in 1–2% yield which was identical in every respect with an authentic sample of strictosidine lactam [1] (**1a**). This was further characterized as its tetra-acetate. Similar treatment of the bark of *Nauclea latifolia* gave large amounts of strictosidine lactam together with traces of a compound with very similar spectra ($R_f$ (1:1:1) 0.71) which was presumably an isomer.

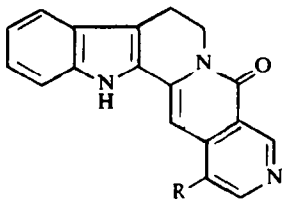
Previous workers had reported the presence in this plant of small quantities of alkaloids with structure (**2a–d**) [2]. In view of the use of ammonia in the extraction procedure and the ease with which dihydrovincoside lactam (**1b**) is converted into dihydroangustine (**2e**)



1 (a) 3α, R = CH = $CH_2$
(b) 3β, R = Et



3



2 (a) R = CH = $CH_2$
(b) R = CHOH.Me
(c) R = H
(d) R = Ac
(e) R = Et

[3], the possibility that the alkaloids (**2a–d**) are artifacts produced from strictosidine lactam during extraction, must be considered [4]. In this connection it was found that strictosidine lactam was slowly converted to a pyridone (**3**) on standing in solution, confirming the ease with which this compound undergoes aerial oxidation [5].

## EXPERIMENTAL

*Nauclea latifolia* heartwood (50 g) was macerated and extracted with MeOH (5 × 200 ml). Removal of the solvent left an orange residue. This was subjected to a counter-current distribution between EtOAc and $H_2O$ (500 ml fractions of each). After three transfers TLC examination showed aq. fractions 1 and 2 and organic fractions 2 and 3 to be essentially one component. The aq. fractions were concd and extracted with EtOAc (7 × 200 ml) and the combined extracts washed with pH 9 buffer (0.025 M Na tetraborate–boric acid) (200 ml). Removal of the solvent gave a yellow solid (0.61 g 1.2%) identical with strictosidine lactam. [TLC (toluene–EtOAc–MeOH, 1:1:1) $R_f$ 0.67; $[\alpha]_D^{20}$ − 77° (MeOH, *c* 0.41), UV $\lambda_{max}^{(MeOH)}$: 290 (3.81), 230 (4.46), nm]. Acetylation ($Ac_2O$–Py gave an orange solid identical in every respect with strictosidine lactam tetra-acetate: TLC (ethyl acetate) $R_f$ 0.7; $[\alpha]_D^{20}$ − 71° ($CHCl_3$ *c* 0.31), UV $\lambda_{max}^{MeOH}$: 290, 229 nm, CD $[\theta]_{265}^{MeOH}$: +2.2 × $10^4$ degree $cm^2$/decimole, IR $\nu_{max}^{CHCl_3}$ 3470, 3270, 1760, 1665, 1650 $cm^{-1}$, NMR ($CDCl_3$) 100 MHz: 1.60 (1H, *s*, NH), 2.50–3.05 (5H, *m*, Aromatic H, C 17-H), 7.96, 8.04, 8.15 and 8.92 (12H, *s*, O.CO.Me), MS *m/e*; $M^+$ 666.2427 (71) (calculated for $C_{34}H_{38}N_2O_{12}$, 666.2425), 378 (7), 335 (12), 331 (16), 319 (16), 289 (7), 265 (16), 236 (5), 235 (7), 169 (100), 144 (14), 143 (19), 127 (21), 115 (9), 109 (57).

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# TEINEMINE AND ISOTEINEMINE, TWO NEW ALKALOIDS FROM *VERATRUM GRANDIFLORUM*

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**Key Word Index**—*Veratrum grandiflorum*; Liliaceae; alkaloids; teinemine; (22*R*, 25*S*)-22,26-epiminocholest-5-ene-3β, 16α-diol; isoteinemine.

The isolation of solanidine [1] and etioline [2] as the main alkaloids from the terrestrial parts of budding *Veratrum grandiflorum* has already been reported. From the same plant material, in addition to veratramine,

two new alkaloids, which were named teinemine (1) and isoteinemine (2) after the Ainu name 'Teine' for *Veratrum*, were obtained from the secondary base fraction after HCl hydrolysis.

Teinemine $C_{27}H_{45}NO_2$ shows in its MS peaks at *m/e* 415, 397, 382, 204, 150, 140, 125, 99, and 98 (base peak), the last 4 corresponding to that of the Me piperidine moiety [3]. The IR spectrum of 1 revealed the presence of an OH group and a secondary amine group at 1065 and 3600 to 3300 $cm^{-1}$, respectively, but no absorption of a C=N double bond was observed at 1660–1640 $cm^{-1}$. The PMR spectrum of 1 displayed two singlets (3H each) at $\delta$ 0.72 and 1 ppm indicative of the C-18 and C-19 angular Me groups of a normal steroid ring system with a $\Delta^5$ double bond [4], two doublets at $\delta$ 0.98 and 1.03 ppm (3H each, $J = 6$ Hz) corresponding to two secondary Me groups at C-21 and C-27, and the signal (doublet) of a C-6 vinyl proton at 5.31 ppm. One of the other protons, a multiplet centered at 3.52 ppm, is associated with a H adjacent to the OH group at C-3, and the other, a multiplet centered at $\delta$ 4.08 ppm, is associated with the $\beta$-proton adjacent to the $\alpha$-OH group at C-16 by comparison with etioline (3) and tetrahydroetioline (4) [5]. After acetylation, 1 formed an *O,O,N*-triacetate, $C_{33}H_{51}NO_5$ showing signals at 2.04, 2.07 (OAc), and 2.10 (*N*-acetyl) ppm in its PMR spectrum and absorption bands at 1730 (OAc) and 1640 (*N*-Ac) $cm^{-1}$ in its IR spectrum.

Catalytic reduction of the 22(*N*)-double bond of etioline (3) in EtOH over $PtO_2$ afforded two dihydroetiolines which, after separation by TLC on Si gel, were obtained in a ratio of 19:1. The physical constants of the main product were identical with those of teinemine (1). Reduction of 1 in HOAc over $PtO_2$ afforded (22*R*)-dihydroteinemine (4) the physical constants of which agreed well with those of (22*R*)-tetrahydroetioline (4) obtained from 3 by the same method. The mp of (22*R*)-dihydroteinemine was not depressed by admixture with (22*R*)-tetrahydroetioline; (22*R*)-dihydroetioline (1) was also converted to 5$\alpha$-solanidan-3-one by oxidation with Kiliani reagent and subsequent reduction over Pd-C [5]. From these results, teinemine was identified as (22*R*, 25*S*)-22,26-epiminocholest-5-ene-3$\beta$, 16$\alpha$-diol.

Isoteinemine (2), $C_{27}H_{45}NO_2$ had similar properties to 1 and corresponded well with the second compound obtained from etioline (3) by catalytic reduction in EtOH over $PtO_2$. Therefore, the structure of 2 was postulated as (22*S*, 25*S*)-22,26-epiminocholest-5-ene-3$\beta$, 16$\alpha$-diol. The small amounts of 2 isolated prevented any further chemical studies.

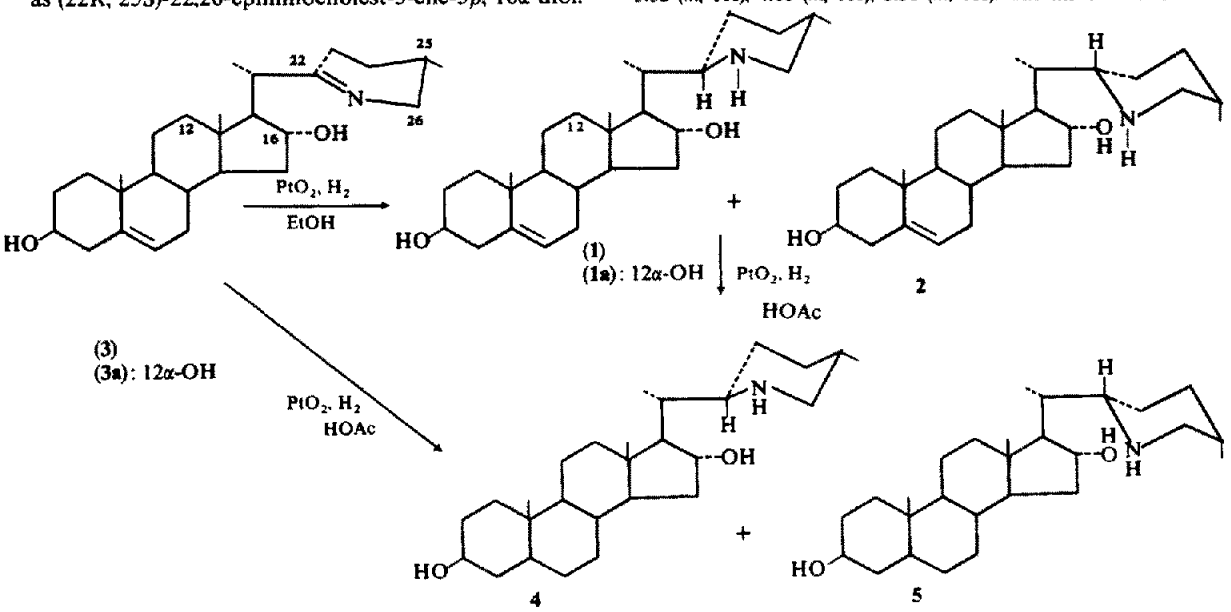
Recently, Ito *et al.* [6] identified baikeine (1a) (22*R*, 25*S*)-22,26-epiminocholest-5-ene-3$\beta$, 12$\alpha$, 16$\alpha$-triol and Kaneko *et al.* [7] hakurirodine (3a) (25*S*)-22,26-epiminocholesta-5,22(*N*)-diene-3$\beta$, 12$\alpha$, 16$\alpha$-triol from *Veratrum*. These two groups of 22,26-epiminocholestene derivatives, either etioline (3) and teinemine (1) or hakurirodine (3a) and baikeine (1a), appear to have an important role in the biosynthesis of solanidine or rubijervine, respectively, in the *Veratrum* plant.

## EXPERIMENTAL

Mps are uncorr. IR spectra were taken in Nujol mulls, optical rotations in $CHCl_3$ soln and PMR spectra at 100 MHz in $CDCl_3$ using TMS as internal standard. TLC was carried out on Si gel $HF_{254}$ and $Al_2O_3$ (neutral II-III) was used for column chromatography.

*Isolation of crude alkaloid.* Terrestrial parts of budding *V. grandiflorum* (Max.) Loesen harvested in early April at Teine, Hokkaido, Japan, were dried and extracted with ammoniacal MeOH–$CHCl_3$ (2:3) to yield 140 g of crude glycoside. Crude alkaloid (32.6 g) was obtained from this glycoside after HCl hydrolysis, as described previously [1]. The alkaloid mixture was separated in to the secondary and tertiary base fractions by the method of ref. [8]. The secondary base fraction (9.7 g) was further separated by chromatography over 30 vol. of $Al_2O_3$ and elution with 10 or 20% $Me_2CO$ in $C_6H_6$.

*Teinemine (1) and isoteinemine (2).* An aliquot (100 mg) of the fraction obtained by elution with 20% $Me_2CO$ in $C_6H_6$, containing in addition to 1 traces of 3 and 2, was further purified by preparative-TLC (*n*-hexane–EtOH–$Et_2NH$; 9:0.75:0.75, cyclohexane–EtOAc–MeOH; 2:2:1). 1 was crystallized from $Me_2CO$ to yield 77.7 mg of plates mp 204–209°; $[\alpha]_D^{20}$ − 35.8° (*c* 1.19, $CHCl_3$); elemental analysis, calcd. for $C_{27}H_{45}NO_2$: C, 78.02; H, 10.91; N, 3.37; found: C, 77.99; H, 10.94; N, 3.44; MS *m/e* 415 ($M^+$), 397, 382, 204, 162, 150, 140, 125, 99, 98 (100); $\nu_{max}$ 3600, 3400–3300, 1090, 1065 $cm^{-1}$; PMR 0.72 (*s*, 3H), 1 (*s*, 3H), 0.98 (*d*, $J = 6$ Hz, 3H), 1.03 (*d*, $J = 6$ Hz, 3H), 3.52 (*m*, 1H), 4.08 (*m*, 1H), 5.31 (*m*, 1H). The minor alkaloid 2



after the purification of 1 was crystallized from $Me_2CO$ yielding 2.8 mg of flakes mp 217–220°; $\nu_{max}$ 3600, 3500–3200, 1060 $cm^{-1}$; MS, *m/e*: 415 ($M^+$), 397, 382, 204, 150, 140, 99, 98 (100); PMR 0.73 (*s*, 3H), 1 (*s*, 3H), 0.83 (*d*, *J* = 6 Hz, 3H), 1.07 (*d*, *J* = 6 Hz, 3H), 3.49 (*m*, 1H), 4.09 (*m*, 1H), 5.35 (*m*, 1H).

*Acetylation of* 1. Twenty-five mg of 1 after acetylation in the usual manner gave 24.9 mg of crystals, mp 147–148.5°; $\nu_{max}$ 1730, 1640, 1250 $cm^{-1}$; MS, *m/e*: 541 ($M^+$), 498, 481, 438, 421, 378, 140 (100), 99, 81; PMR 0.86 (*s*, 3H), 1.02 (*s*, 3H) 0.88 (*d*, *J* = 6 Hz, 3H), 0.92 (*d*, *J* = 6 Hz, 3H), 2.04 (*s*, 3H), 2.07 (*s*, 3H), 2.1 (*s*, 3H) 4.56 (*m*, 1H), 5.14 (*m*, 1H), 5.36 (*m*, 1H); elemental analysis, calcd. for $C_{33}H_{51}NO_5$: C, 73.96; H, 9.49; N, 2.59; found: C, 73.22; H, 9.56; N, 2.63.

*Hydrogenation of* 3. A soln of 3 (159 mg) in 4 ml of EtOH was reduced with 167 mg of $PtO_2$ catalyst in $H_2$ at 25°. It consumed 1 mol equivalent of $H_2$ after 18.5 hr. The reaction mixture was extracted with $Et_2O$ after removal of catalyst. The product was purified by repeated preparative TLC (*n*-hexane–EtOH–$Et_2NH$; 9:0.75:0.75, cyclohexane–EtOAc–MeOH; 2:2:1) and the main product (1) was crystallized from $Me_2CO$, yield 130.5 mg, mp 203.5–208°. Elemental analysis, calcd. for $C_{27}H_{45}NO_2$: C, 78.02, H, 10.91; N, 3.37; found: C, 77.98; H, 10.93; N, 3.60; MS, *m/e*: 415 ($M^+$), 397, 204, 162, 150, 140, 99, 98 (100); PMR 0.71 (*s*, 3H), 1 (*s*, 3H), 0.97 (*d*, *J* = 6 Hz, 3H), 1.03 (*d*, *J* = 6 Hz, 3H), 3.52 (*m*, 1H), 4.06 (*m*, 1H), 5.32 (m, 1H), $\nu_{max}$ 3600, 3400–3300, 1090, 1065 $cm^{-1}$; $[\alpha]_D^{17.5}$ − 34.9° (*c* 1.19, $CHCl_3$). The minor product 2 was crystallized from $Me_2CO$, yield 23.9 mg, mp 217.5–222.5°. MS, *m/e*: 415 ($M^+$), 397, 382, 204, 150, 140, 99, 98 (100); PMR 0.73 (*s*, 3H), 1 (*s*, 3H), 0.82 (*d*, *J* = 6 Hz, 3H), 1.06 (*d*, *J* = 6 Hz, 3H), 3.53 (*m*, 1H), 4.11 (*m*, 1H), 5.35 (*m*, 1H); $\nu_{max}$ 3600, 3500–3200, 1060 $cm^{-1}$.

*Conversion of teinemine to solanidan-3-one.* A soln of 1 (250 mg) in 30 ml of HOAc was hydrogenated with $PtO_2$ catalyst (300 mg) under $H_2$ at 20°. After 5 hr, the absorption of gas ceased with the uptake of 1 mol equivalent of $H_2$. The product was worked up in the usual manner to give 203 mg of dihydroteinemine (tetrahydroetioline) (4), mp 202–204°; MS, *m/e*: 415 ($M^+$ − 2), 399, 397, 384, 382, 280, 204, 150, 98 (100); PMR 0.65 (*s*, 3H), 0.76 (*s*, 3H), 0.92 (*d*, J = 6 Hz, 3H), 1 (*d*, J = 6 Hz, 3H), 3.53 (*m*, 1H), 4 (*t*, J = 6 Hz, 1H). To a soln of 4 (203 mg) in 65 ml of $Me_2CO$ was added dropwise, 2 ml of Kiliani's soln and the mixture allowed to stand for 30 min at room temp. The reaction mixture was made alkaline with aq. $K_2CO_3$ and extracted with $Et_2O$. The oily product was hydrogenated with 0.1 g of 10% Pd-C in 30 ml of EtOAc–MeOH (1:3) under $H_2$. After the uptake of 1 mol equivalent of $H_2$ in 3 hr, the product was worked up in the usual manner to give 200 mg of an oily mass, which was purified by TLC (cyclohexane–EtOAc; 4:1) and crystallized from $Me_2CO$ to yield flakes (165 mg), mp 210–214°, MS, *m/e* 397 ($M^+$), 204 150 (100). It showed no depression on admixture with an authentic specimen of solanidan-3-one (mp 211–213°) obtained from solanidine [5]. The spectral data of both samples were also identical.

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