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ISOLATION AND THE STRUCTURES OF TWO NEW ALKALOIDS, PETASITENINE AND NEOPETASITENINE FROM PETASITES JAPONICUS MAXIM.

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Two new pyrrolizidine alkaloids revealing strong hepatotoxic activity were isolated from Petasites japonicus Maxim., and their structures were deduced to be (3) and (4), respectively based on chemical and spectral evidence.

Previously, one of the authors (I.H.) reported the carcinogenic activity of Petasites japonicus Maxim. (Fukinotoh in Japanese).[1] In connection with the carcinogenic activity, we have investigated the chemical constituents of this plant.

We herein describe the isolation of two new pyrrolizidine alkaloids showing strong hepatotoxic activity from Petasites japonicus Maxim. and their structural elucidation. The alkaloidal fraction obtained from the ethanol extract of the dried powdered plant was chromatographed first on silicic acid with methanol and subsequently on aluminum oxide with chloroform, affording two alkaloids: petasitenine (3),[2] mp 129 - 130° (benzene - n-hexane), $C_{19}H_{27}O_7N$, $[\alpha]_D^{19}$ + 44° (c 1.08, EtOH), ir($CHCl_3$) 3500, 1740(shoulder), 1730, 1660, 1610 cm^{-1}; mass 381 (M^+), 366, 353, 337, 322, 310, 309, 294, 266; nmr (Table): neopetasitenine (4),[2] amorphous powder [HCl salt,[2] mp 212°(dec); picrate,[2] mp 223 - 225°], $C_{21}H_{29}O_8N$, $[\alpha]_D^{19}$ + 49° (c 1.19, EtOH), ir($CHCl_3$) 1755 (shoulder), 1740, 1660, 1610 cm^{-1}; mass 423 (M^+), 408, 380, 379, 364, 336, 319, 304, 264; nmr (Table). It was suggested that neopetasitenine was the O-acetate of petasitenine by the spectral data: indeed, petasitenine was converted to neopetasitenine by acetylation (Ac_2O - Et_3N - 4-dimethylaminopyridine, room temp.).

Comparison of the ir, nmr, and mass spectra of petasitenine with those[3] of various pyrrolizidine alkaloids suggests that petasitenine is a macrocyclic diester of otonecine type amino alcohol. The molecular formula of petasitenine (3) is identical with that of otosenine (1),[4,5] one of the representative macrocyclic diester pyrrolizidine alkaloids of otonecine type. Although the thin layer chromatographic behavior of petasitenine (3) differs from that of otosenine (1), the spectral properties of both alkaloids are quite similar. The characteristic signals in the nmr spectrum of petasitenine (3) correspond well to those of otosenine (1) as shown in the table, and further, the mass spectrum of petasitenine (3) is indistinguishable from that of otosenine (1). The close resemblance of these spectral properties strongly suggests that petasitenine (3) is a diastereomer of otosenine (1). In order to confirm this inference, the following degradation experiments were performed.

Acid hydrolysis of petasitenine (3) (10% HCl, reflux, 45 hr) afforded an amino alcohol, which was isolated as the hydrochloride (gum). The nmr spectrum of the amino alcohol

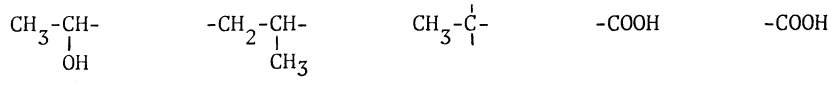
Table. NMR Spectral Data (δ in ppm, 100 MHz, $CDCl_3$)

compound	H-2	H-3	H-7	H-9	N-CH_3	H-11	H-14	H-18	H-19
1	6.14 m	3.40 br.s	5.12 m	5.48, 4.37 AB q 11.0	2.12 s	1.35 s	1.16 d 6.0	2.98 q 5.5	1.25 d 5.5
3	6.16 m	3.58, 3.30 AB q 18.0	5.19 m	5.48, 4.34 AB q 12.0	2.08 s	1.35 s	1.07 d 6.5	3.04 q 5.0	1.46 d 5.0
4[a)]	6.19 t 2.0	3.52, 3.28 AB q 17.0	5.08 m	5.31, 4.36 AB q 11.0	2.08 s	1.71 s	1.09 d 6.5	3.04 q 5.0	1.47 d 5.5

a) AcO : 2.08 (3H, s)

hydrochloride was superimposable with that[6] of otonecine (5) hydrochloride. Hydrogenation of petasitenine (3) (H_2/PtO_2, HCl - EtOH, room temp.) followed by hydrolysis (10% NaOH, room temp.) gave dihydrodeoxyotonecine (6),[7] a derivative known to be suitable for identification of otonecine, by spectral comparison and optical rotation. From these results the necine part of petasitenine (3) was shown to be otonecine (5).

Alkaline hydrolysis of petasitenine (3) [10% $Ba(OH)_2$, reflux, 1 hr] yielded a dicarboxylic acid, petasitenecic acid (10),[2] $C_{10}H_{16}O_6$, mp 178 - 180°, which contains all of carbon, hydrogen, and oxygen of the necic acid moiety of the alkaloid, assuming that simple hydrolysis of the diester part of the alkaloid took place. It was shown by direct comparison (mixed mp and ir spectra) that petasitenecic acid (10) was not identical with jaconecic acid (7)[8,9] ($C_{10}H_{16}O_6$) obtained by alkaline hydrolysis of pyrrolizidine alkaloids such as otosenine (1)[4a-c,10] and jacobine (17).[8a-f] The dimethyl ester (11)[2,11] prepared by methylation of petasitenecic acid (10) by ethereal diazomethane was converted to a diester acetate (12)[2,12] by acetylation (Ac_2O - py, room temp.). Oxidation of the dimethyl ester (11) (CrO_3 - py, room temp.) afforded a keto diester (13),[2,13] which was shown to be different from dimethyl jaconecate ketone (9)[20] obtained by oxidation of dimethyl jaconecate (8).[8b] From the nmr spectral data[11,12,13] of (11), (12), and (13), the presence of the following groups, I, II, III, IV, and V in (10) was indicated.



(I) (II) (III) (IV) (V)

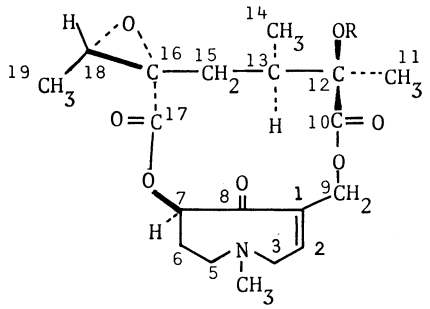
Although there remained one carbon and one oxygen to be characterized in petasitenecic acid (10), they were assigned to a quaternary carbon and a cyclic ethereal oxygen, respectively, considering the molecular formula. Definitive information on the carbon skeleton of petasitenecic acid (10) was secured by the oxidation of the acid (10) itself (CrO_3 - py, room temp.) to give a γ-lactone (14).[14] This product was found to be identical with the γ-lactone (14)[8e,8f,15] obtained by the oxidation[16] of jaconecic acid (7) by spectral and thin layer chromatographic comparison together with optical rotation. Based on the partial structures (I - V) and the formation of the γ-lactone (14), the planar structure of petasitenecic acid (10) was proved to be the same as that of jaconecic acid (7). Further, it is evident that stereochemistry at C-12 and C-13 in petasitenecic acid (10) is identical with that in jaconecic acid (7). Since petasitenecic acid (10) is not identical with jaconecic acid (7), and the keto diester (13) differs from dimethyl jaconecate ketone (9) (vide ante), it is concluded that petasitenecic acid

(10) is diastereomeric with jaconecic acid (7) at least at C-16.[17] Considering the mode[18] of formation of petasitenecic acid (10) from petasitenine (3), the structure of the necic acid in petasitenine is represented by (16).

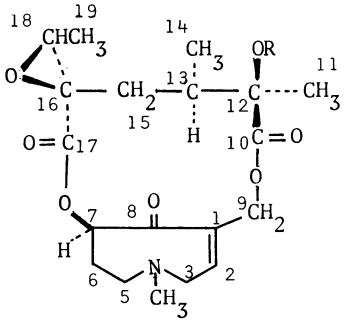
Thus the structures of the necine and the necic acid in petasitenine were clarified.

Mass spectra provided valuable evidence on the mode of two ester linkages in petasitenine (3). The characteristic fragmentation patterns of otosenine (1) and the related alkaloids were analyzed and correlated with their structures including the allylic ester moiety.[3,19] Since the mass spectra of petasitenine (3) and neopetasitenine (4) were indistinguishable from those of otosenine (1) and florosenine (otosenine O-acetate) (2),[19b] respectively, the mode of two ester linkages in petasitenine (3) was deduced to be the same as that in otosenine (1). Therefore, the structures of petasitenine and neopetasitenine are represented by (3) and (4), respectively.

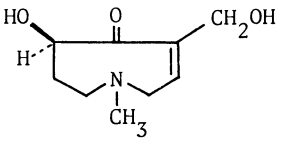
Acknowledgements. Financial support from the Ministry of Education, Science, and Culture (Grant-in-Aid for Cancer Research) is gratefully acknowledged. One of the authors (K.Y.) wishes to thank the Kurata Foundation, the Yamaji Foundation, and Takeda Science Foundation for support of this work.



1 : R = H (otosenine)
2 : R = Ac (florosenine)



3 : R = H (petasitenine)
4 : R = Ac (neopetasitenine)



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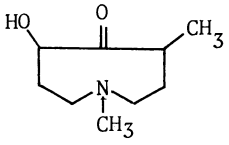

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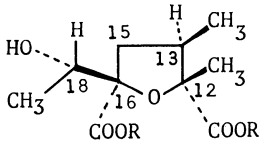
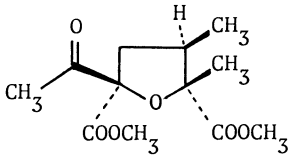


7 : R = H
8 : R = CH_3



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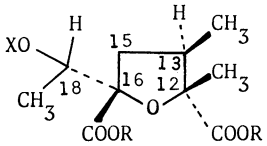
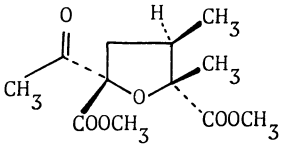
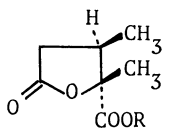


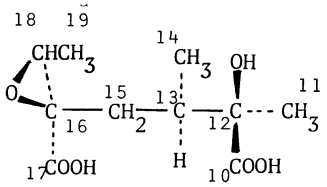
10 : R = X = H
11 : R = CH_3, X = H
12 : R = CH_3, X = Ac



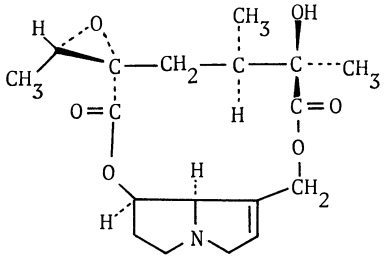
13



14 : R = H
15 : R = CH_3



16



17 (jacobine)

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2. Satisfactory microanalyses or high resolution mass spectral data were obtained.
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5. We are very grateful to Prof. M. P. Cava (Univ. Pennsylvania, U.S.A.), Dr. C. C. J. Culvenor (C.S.I.R.O., Australia), and Prof. F. Šantavý (Palacký Univ., Czechoslovakia) for providing us with valuable samples of otosenine.
6. Otonecine hydrochloride was prepared from otosenine: cf. C. K. Atal, C. C. J. Culvenor, R. S. Sawhney, and L. W. Smith, Aust. J. Chem., 20, 805 (1967).
7. Dihydrodeoxyotonecine was prepared from otosenine: cf. C. C. J. Culvenor, G. M. O'Donovan, and L. W. Smith, Aust. J. Chem., 20, 801 (1967). We thank Dr. C. C. J. Culvenor for providing us with ir and nmr spectral copies of dihydrodeoxyotonecine.
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   e) R. B. Bradbury and S. Masamune, J. Amer. Chem. Soc., 81, 5201 (1959).
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   g) J. Fridrichsons, A. McL. Mathieson, and J. Sutor, Tetrahedron Lett., No.26, 18 (1960).
9. We are greatly indebted to Prof. S. Masamune (Univ. Alberta, Canada) for generous gift of jaconecic acid.
10. N. I. Koretskaya, A. V. Danilova, and L. M. Utkin, Zh. Obshch. Khim., 32, 1339 (1962).
11. ir($CHCl_3$) 3600, 3520, 1744 cm^{-1}; nmr($CDCl_3$, 100 MHz) δ1.02 (3H, d, J=6.0 Hz, $C\underline{H}_3CH$-), 1.13 (3H, d, J=6.0 Hz, $CH_3C\underline{H}(OH)$-), 1.35 (3H, s, CH_3-C-), 1.60 (1H, br.s, OH), 2.0 - 2.8 (3H, pattern of ABC type, $-C\underline{H}_2-C\underline{H}(CH_3)$-), 3.75 (3H, s, $COOCH_3$), 3.77 (3H, s, $COOCH_3$), 4.08 (1H, q, J=6.0 Hz, $CH_3C\underline{H}(OH)$-); mass 260(M^+).
12. ir($CHCl_3$) 1745 cm^{-1}; nmr ($CDCl_3$, 100 MHz) δ 1.05 (3H, d, J=6.0 Hz, $C\underline{H}_3$-CH-), 1.26 (3H, d, J=6.0 Hz, $C\underline{H}_3CH(OAc)$-), 1.33 (3H, s, CH_3-C-), 2.03 (3H, s, AcO), 1.9 - 2.8 (3H, pattern of ABC type, $-C\underline{H}_2-C\underline{H}(CH_3)$-), 3.74 (3H, s, $COOCH_3$), 3.76 (3H, s, $COOCH_3$), 5.27 (1H, q, J=6.0 Hz, $CH_3C\underline{H}(OAc)$-); mass 302 (M^+).
13. ir($CHCl_3$) 1750, 1720(shoulder) cm^{-1}; nmr($CDCl_3$, 100 MHz) δ 1.06 (3H, d, J=6.5 Hz, $C\underline{H}_3CH$-), 1.42 (3H, s, CH_3-C-), 2.36 (3H, s, CH_3-CO-), 2.88 (1H, dd, J=13.0, 6.0 Hz, -HCH-$CH(CH_3)$-), 2.00 (1H, dd, J=13.0, 9.5 Hz, -HCH-$CH(CH_3)$-), 2.40 (1H, m, $-CH_2-C\underline{H}(CH_3)$-), 3.72 (3H, s, $COOCH_3$), 3.78 (3H, s, $COOCH_3$); mass 258 (M^+).
14. The γ-lactone (14) was purified and characterized as the methyl ester (15).
15. The structure of (14) including absolute configuration was unambiguously established.[8f,8g]
16. Originally, the oxidation[(7) → (14)] was performed using lead tetraacetate.[8e,8f] In the present work this oxidation was found to be effected also by chromic acid in pyridine. For mechanistic interpretation of the reaction, see ref. 8d and 8e.
17. Since the configuration at C-18 in petasitenecic acid (10) remained unsettled, it is unknown whether petasitenecic acid (10) is diastereomeric with jaconecic acid (7) regarding C-18 or not.
18. Formation of petasitenecic acid (10) from petasitenine (3) is quite analogous to that of jaconecic acid (7) from the alkaloids such as otosenine (1) and jacobine (17), and the mechanism of the reaction [intramolecular displacement at C-16 by tertiary OH on C-12 (inversion at C-16)] was well established.[8d,8e,3]
19. a) C. K. Atal, K. K. Kapur, C. C. J. Culvenor, and L. W. Smith, Tetrahedron Lett., 537 (1966).
    b) M. P. Cava, K. V. Rao, J. A. Weisbach, R. F. Raffauf, and B. Douglas, J. Org. Chem., 33, 3570 (1968).
20. ir($CHCl_3$) 1750, 1720(shoulder) cm^{-1}; nmr($CDCl_3$, 100 MHz) δ 1.04 (3H, d, J=6.0 Hz), 1.41 (3H, s), 2.34 (3H, s), 1.8 - 2.9 (3H, pattern of ABC type), 3.75 (3H, s), 3.80 (3H, s); mass 258 (M^+).

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