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PETASININE AND PETASINOSIDE, TWO MINOR ALKALOIDS POSSESSING A NEW NECINE ISOLATED FROM PETASITES JAPONICUS MAXIM.

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Carcinogenicity of the young flower stalks of Petasites japonicus Maxim. was reported by one of us (I.H.).[1] In the course of a search for the carcinogen(s) of this plant two pyrrolizidine alkaloids, petasitenine and neopetasitenine, were isolated and their structures were determined,[2,3,4] the former alkaloid being proved to be a carcinogen.[5] In further scrutiny of the alkaloidal components of the same source we have isolated two new alkaloids, petasinine (1) and petasinoside (3), the structures of which are described in this paper.

The alkaloidal fraction obtained from the ethanol extract of the dried powdered plant was chromatographed first on silicic acid (MeOH) and subsequently separated by preparative TLC on alumina (10% MeOH - $CHCl_3$), giving petasinine (1) (0.0008% dry weight) and petasinoside (3) (0.0005% dry weight). The pure sample of petasinine was obtained by purification using HPLC (MicroPak NH_2-10 with 2.5% i-PrOH - CH_2Cl_2).

Petasinine (1),[6] amorphous powder [phenylurethane,[6,7] mp 168 - 171° (benzene)], $C_{13}H_{21}O_3N$, $[\alpha]_D^{25}$ +16° (c 2.5, EtOH); IR ($CHCl_3$) 3460, 1698, 1640 cm^{-1}; NMR ($CDCl_3$) δ 1.90 (3H, br s), 2.03 (3H, dq, J = 7.0, 1.0 Hz), 3.68 (2H, m), 5.33 (1H, t, J = 4.0 Hz), 6.15 (1H, br q, J = 7.0 Hz); mass 239 (M^+), 140, 139, 83.

Petasinoside (3),[8] amorphous powder, $C_{28}H_{37}O_9N$, $[\alpha]_D^{25}$ -38° (c 1.1, EtOH); IR ($CHCl_3$) 3340, 1703, 1630, 1604, 1580, 1505 cm^{-1}; NMR ($CDCl_3$) δ 1.25 (3H, d, J = ca. 6 Hz),[9] 1.87 (3H, br s), 1.99 (3H, dq, J = 7.0, 1.0 Hz), 5.37 (1H, br t, J = 4.0 Hz), 5.53 (1H, br s), 6.10 (1H, br q, J = 7.0 Hz), 6.27 and 7.59 (2H, ABq, J = 16.0 Hz), 6.9 - 7.5 (4H, m, A_2B_2 type).

Petasinoside (3) was fully characterized as its triacetate (4) obtained on acetylation (Ac_2O - pyridine, 25°) followed by HPLC (MicroPak Si-5 with 5% i-PrOH - CH_2Cl_2) purification.

Petasinoside triacetate (4),[6] amorphous powder, $C_{34}H_{43}O_{12}N$, $[\alpha]_D^{25}$ -52° (c 0.65, EtOH); IR ($CHCl_3$) 1746, 1708, 1630, 1603, 1582, 1510 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (3H, d, J = 6.5 Hz), 1.90 (3H, br s), 1.96 (3H, br d, J = 7.0 Hz), 2.05, 2.08, and 2.21 (3H each, s), 3.98 (1H, m), 4.43 (2H, d, J = 8.0 Hz), 5.18 (1H, dd, J = 10.0, 10.0 Hz), 5.3 - 5.7 (4H, complex m), 6.12 (1H, br q, J = 7.0 Hz), 6.33 and 7.65 (2H, ABq, J = 16.0 Hz), 7.0 - 7.6 (4H, m, A_2B_2 type); mass 657 (M^+), 558, 273, 222, 153, 83.

Structure of petasinine (1). Acetylation of 1 (Ac_2O - pyridine, 25°) afforded the monoacetate (2).[6,10] In the NMR spectra a two-proton multiplet at δ 3.68 in 1 appeared at δ 4.30 in 2, revealing the presence of a primary hydroxyl group in 1. Alkaline hydrolysis [17% KOH in H_2O - MeOH (1:2), 25°] of 1 afforded angelic acid, which was identified as the p-bromophenacyl ester (mp 66 - 67°), and a new necine, petasinecine (5):[6] mp 132 - 134° (acetone), $C_8H_{15}O_2N$, $[\alpha]_D^{25}$ -20° (c 0.25, EtOH); IR (KBr) 3275 cm^{-1}; NMR ($CDCl_3$) δ 1.5 - 3.7 (12H, complex m), 3.89 (1H, dd, J = 11.0, 6.0 Hz), 3.94 (1H, dd, J = 11.0, 8.0 Hz), 4.54 (1H, dt, J = 5.5, 5.0 Hz); mass 157 (M^+), 140, 98, 83 (base peak). On acetylation (Ac_2O - pyridine, 20°) petasinecine (5) gave the diacetate (6),[6,11] (amorphous powder). Comparison of the NMR spectra of 5 and 6 clearly indicates that two hydroxyl groups in 5 are primary and secondary, and further that the secondary hydroxyl group forms the ester linkage with angelic acid in 1, if one takes account of the NMR spectral data of 1 and 2 (vide ante). Considering the molecular formula and the functional groups [$-CH_2OH$, $-\overset{|}{C}HOH$, $-\overset{|}{N}-$ (tertiary amine)], petasinecine (5) was deduced to be a saturated 1-hydroxymethylpyrrolizidine with a secondary hydroxyl group. Since petasinecine (5) does not show the chemical and spectral properties of a carbinolamine, the secondary hydroxyl group must be in one of the three possible positions, C-2, C-6, and C-7, in the 1-hydroxymethylpyrrolizidine nucleus (cf. 5). Mass spectrometry was valuable in defining the location of the secondary hydroxyl group in 5. The mass spectrum of 5 showed the prominent fragment peaks at m/e 83 (base peak) and 98: it is known that a base peak of m/e 83 together with a strong peak at m/e 98 is indicative of the unsubstituted ring A of the 1-hydroxymethylpyrrolizidine nucleus, whereas the 1-hydroxymethylpyrrolizidine having a hydroxyl group at C-6 or C-7 exhibits a base peak of m/e 82 with only one other major peak of m/e 113.[12] Thus the planar structure of petasinecine (5) was deduced to be 2-hydroxy-1-hydroxymethylpyrrolizidine, and it must be stereoisomeric with macronecine (2β-hydroxy-1β-hydroxymethyl-8β-pyrrolizidine).[12,13] In connection with the structure of macronecine, four possible stereoisomers of 2-hydroxy-1-hydroxymethylpyrrolizidine were previously synthesized in racemic forms and the stereochemistry of each isomer was unambiguously determined by Culvenor and Aasen.[13] The spectral (solution IR, NMR, and mass) and TLC properties of petasinecine diacetate (6) was found to be identical with those of the diacetate of (±)-2β-hydroxy-1β-hydroxymethyl-8α-pyrrolizidine, one of the stereoisomers synthesized by Culvenor and Aasen.[13,14] Therefore the structure of petasinine is represented by 1.

Structure of petasinoside (3). Methanolysis [NaOMe (45 molar equiv) - MeOH, 25°] of petasinoside triacetate (4) afforded petasinecine (5), methyl angelate, and a methyl ester (7)[6,15] [mp 148 - 150° (benzene - $CHCl_3$), $C_{16}H_{20}O_7$]. The structure of the methyl ester was deduced to be 7, based on the spectral data of 7 and its triacetate (8)[6,16] [mp 136 - 137° (benzene - hexane), $C_{22}H_{26}O_{10}$, $[\alpha]_D^{25}$ -95° (c 0.42, EtOH)] obtained by acetylation (Ac_2O - pyridine, 25°) of 7. This inference was confirmed by the synthesis of 8: methyl trans-p-coumarate was condensed (p-TsOH - toluene, reflux) with L-rhamnose tetraacetate (a mixture of two anomers) to give a single product [mp 135 - 137° (benzene - hexane)], which was proved to be the triacetate (8) obtained on methanolysis of 4 by the mixed mp and spectral (IR, NMR, mass, $[\alpha]_D$) comparison. The α-configuration of the glycosidic linkage in 8 was shown by

the ^{13}C-H coupling constant ($^{1}J[^{13}CH(1)]$ = 174 Hz) of the anomeric carbon.[17,18] The structure including stereochemistry of the methyl ester (7) was thus determined. From the findings described above, petasinoside triacetate (4) was shown to be petasinecine (5) esterified with angelic acid and the acid (9). The location of the two esterifying acids in 4 was established by transformation of petasinine (1) to 4 as follows. The acid (9)[19] obtained by alkaline hydrolysis of 8 followed by acetylation was converted [$(COCl)_2$ - DME, 20°] to the acid chloride, which was reacted (4-dimethylaminopyridine - DME, 20°) with petasinine (1) to give petasinoside triacetate (4).[20] Thus the structure of petasinoside was determined to be 3.

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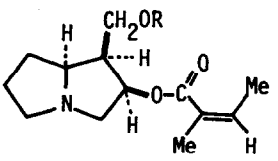
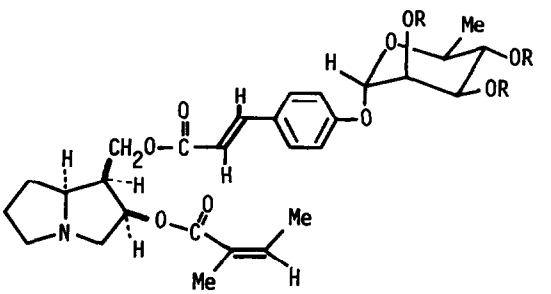
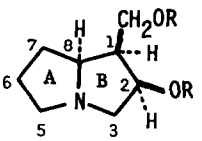


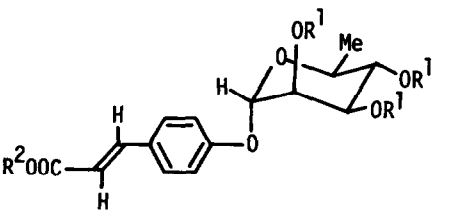
1, R = H (petasinine)
2, R = Ac



3, R = H (petasinoside)
4, R = Ac



5, R = H (petasinecine)
6, R = Ac



7, R^1 = H, R^2 = Me
8, R^1 = Ac, R^2 = Me
9, R^1 = Ac, R^2 = H

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6. Satisfactory microanalyses or high resolution mass spectral data were obtained.
7. Phenylurethane: IR ($CHCl_3$) 1725, 1640, 1600 cm^{-1}; NMR ($CDCl_3$) δ 1.90 (3H, br s), 2.03 (3H, dq, J = 7.0, 1.0 Hz), 4.38 (2H, d, J = 8.0 Hz), 5.45 (1H, t, J = 4.0 Hz), 6.16 (1H, br q, J = 7.0 Hz); mass 358(M^+), 259, 222, 122, 121, 120, 83.
8. The molecular formula of petasinoside (3) was determined by consideration of the physical and spectral properties of petasinoside triacetate (4). No molecular ion peak was observed in the mass spectrum of 3.
9. This doublet could not clearly be observed because of overlapping with other singnals arising from impurities.
10. (2): IR ($CHCl_3$) 1735, 1703, 1645 cm^{-1}; NMR ($CDCl_3$) δ 1.88 (3H, br s), 1.98 (3H, br d, J = 7.0 Hz), 2.03 (3H, s), 4.30 (2H, m), 5.27 (1H, t, J = 4.0 Hz), 6.08 (1H, br q, J = 7.0 Hz); mass 281 (M^+), 222, 182, 122, 121, 120, 108, 83.
11. (6): IR ($CHCl_3$) 1730 cm^{-1}; NMR ($CDCl_3$) 2.04 (2 x 3H, s), 4.21 (1H, dd, J = 11.0, 7.0 Hz), 4.30 (1H, dd, J = 11.0, 8.0 Hz), 5.26 (1H, br t, J = 5.0 Hz); mass 241 (M^+), 182, 122, 121, 120, 108, 83.
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15. (7): IR ($CHCl_3$) 3360, 1700, 1630, 1605, 1580, 1510 cm^{-1}; NMR (methanol-d_4) δ 1.22 (3H, d, J = 5.0 Hz), 3.78 (3H, s), 5.50 (1H, d, J = 2.0 Hz), 6.40 and 7.64 (2H, ABq, J = 16.0 Hz) 6.95 - 7.65 (4H, m, A_2B_2 type); mass 324(M^+), 293, 275, 220, 208, 179, 178.
16. (8): IR ($CHCl_3$) 1745, 1708, 1635, 1606, 1580, 1510 cm^{-1}; NMR (benzene-d_6) δ 1.11 (3H, d, J = 6.0 Hz), 1.66, 1.69, and 1.78 (3H each, s), 3.54 (3H, s), 3.92 (1H, dq, J = 10.0, 6.0 Hz), 5.30 (1H, d, J = 2.0 Hz), 5.52 (1H, dd, J = 10.0, 10.0 Hz), 5.70 (1H, dd, J = 4.0, 2.0 Hz), 5.84 (1H, dd, J = 10.0, 4.0 Hz), 6.36 and 7.76 (2H, ABq, J = 16.0 Hz), 6.65 - 7.15 (4H, m, A_2B_2 type); mass 450 (M^+), 418, 352, 324, 273.
17. K. Bock and C. Pedersen, J. Chem. Soc. Perkin II, 293 (1974).
18. The ^{13}C-H coupling constant of the anomeric carbon was determined concerning the both anomers of rhamnose tetraacetate: 176 Hz for the α-anomer; 163 Hz for the β-anomer. It is known that in 1-O-acetyl compounds the ^{13}C-H(1) coupling constant is increased by ca. 5 Hz in both anomers relative to that of the methyl glycosides.[17]
19. The structure assignment for this compound was based on IR, NMR, and mass spectral evidence.
20. There is a possibility that the migration of the angeloyl group in petasinine (1) occurred from C-2 to C-9 and subsequently the secondary hydroxyl group at C-2 was esterified with the acid chloride of 9, resulting in the formation of petasinoside triacetate (4). This possibility was excluded by the fact that no such acyl migration took place under the reaction conditions of converting 1 to 4.

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