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Steroidal Alkaloids of *Marsdenia rostrata*. III* Rostratine and Dihydrorostratine

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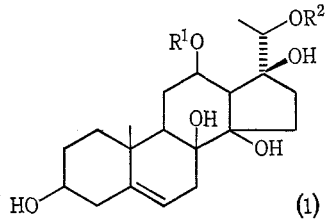
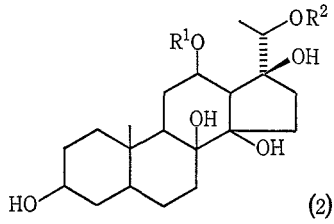
Abstract

The ester alkaloids rostratine and dihydrorostratine were isolated and characterized. On the basis of the mass spectral fragmentation pattern their acetate ester groups were assigned to the C 12 and the nicotinate ester groups to the C 20 hydroxyl groups of the steroid skeleton.

We have previously assigned part-structures (1 and 2; R^1 or R^2 = acetyl, R^2 or R^1 = nicotinoyl) to rostratine and dihydrorostratine, the constituents of the unseparated alkaloid mixture obtained from the Toonumbar collection of *M. rostrata*.[1] This paper describes the isolation, characterization and structure elucidation of the two alkaloids.



(1)



(2)

Rostratine (1; R^1 = acetyl, R^2 = nicotinoyl), $C_{29}H_{39}NO_8$, was separated from dihydrorostratine (2; R^1 = acetyl; R^2 = nicotinoyl), $C_{29}H_{41}NO_8$, by thin-layer chromatography on silver nitrate impregnated silica gel. The spectra of the individual alkaloids confirmed the deductions made from the u.v., i.r., mass and n.m.r.[2,3] spectra obtained from the original alkaloid mixture.

Detailed examination of the mass spectral fragmentation pattern of rostratine, where high resolution measurements were made on an MS-9 spectrometer with computerized data acquisition and analysis system, allows us to assign the nicotinoyl group to the C 20 hydroxyl and, consequently, the acetyl group to the C 12 hydroxyl group of the steroid molecule. The base peak at *m/e* 124 ($C_6H_6NO_2$, protonated nicotinic acid) in the mass spectrum of rostratine is probably formed by hydrogen

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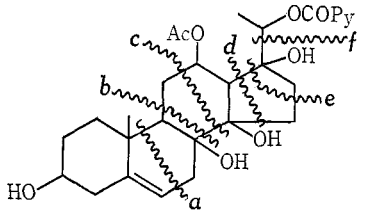
[1] Summons, R. E., Ellis, J., and Gellert, E., *Phytochemistry*, 1972, **11**, 3335.
[2] Sawlewicz, L., Weiss, Ek., and Reichstein, T., *Helv. Chim. Acta*, 1967, **50**, 504, 530.
[3] Schaub, F., Kaufmann, H., Stöcklin, W., and Reichstein, T., *Helv. Chim. Acta*, 1968, **51**, 738.

transfer often observed with esters of aliphatic alcohols. Other prominent peaks at *m/e* 123 ($C_6H_5NO_2$, nicotinic acid), 106 (C_6H_4NO, nicotinoyl), 105 (C_6H_3NO, nicotinoyl−H), and 78 (C_5H_4N, pyridyl), which are also present in the spectrum of nicotinic acid itself, could arise either directly from the molecular ion, *m/e* 529, or from the *m/e* 124 fragment. The same peaks are also present in the low-resolution spectrum of dihydrorostratine. Peaks such as *m/e* 511 ($M^+ - H_2O$), 493 ($M^+ - 2 \times H_2O$), 469 (M^+ − AcOH), 433 (M^+ − AcOH − $2 \times H_2O$), 346 (M^+ − AcOH − nicotinic acid), 328 (M^+ − AcOH − nicotinic acid − H_2O) and 292 (M^+ − AcOH − nicotinic acid − $3 \times H_2O$) are due to loss of combinations of water, acetic and nicotinic acid molecules, while the additional loss of a methyl group gives rise to peaks at *m/e* 454 (469 − Me·), 418 (433 − Me·), 313 (328 − Me·). Similar peaks appear, at two mass units higher, in the spectrum of dihydrorostratine. Retro Dields–Alder fission of ring B[4,5] (which is of course considerably less significant with dihydrorostratine) as shown by dissection *a* generates peaks at *m/e* 138 ($C_9H_{14}O$) and 120 (C_9H_{12}; 138 − H_2O) together with *m/e* 373 ($C_{20}H_{23}NO_6$) and 313 (373 − AcOH). This shows clearly that the hydroxyl group at C 3 is not esterified and confirms the partial formulae (1 and 2; R^1 or R^2 = AcO, R^2 or R^1 = nicotinoyl) proposed earlier.



The characteristic fission of ring C of 8,14-steroid diols[5–7] provides peaks significant for the assignment of the ester groups. Peaks at *m/e* 161 (A/B ring fragment − H_2O − H) and *m/e* 289 (C/D ring fragment − AcOH) are formed according to dissection *b*, while cleavage at dissection *c* yields *m/e* 175 (A/B ring fragment − H_2O − H) and *m/e* 276 (C/D ring fragment − AcO). The peaks at *m/e* 166 (289 − 123) and *m/e* 153 (276 − 123) are due to the additional loss of a molecule of nicotinic acid. However, the most important peaks for the assignment of the ester peaks originate from the fission of ring D, i.e. *m/e* 206 (dissection *d*) and *m/e* 193 (dissection *e*), and from the side chain, *m/e* 150 (dissection *f*). Focused metastable measurements from the first field-free region show that *m/e* 206 is a parent of both *m/e* 193 and 150 peaks and is a daughter of *m/e* 469, 289 and 276 peaks confirming the evidence for assigning structure (1; R^1 = Ac, R^2 = nicotinoyl) to rostratine and, consequently, structure (2; R^1 = Ac, R^2 = nicotinoyl) to dihydrorostratine.

Experimental

The alkaloid mixture from the Toonumbar collection of *M. rostrata*[1] was chromatographed on silver nitrate impregnated silica gel as described for rostratamine.[8] The R_F values quoted refer to the same system. Extraction of the appropriate zones on the chromatogram gave:

[4] Kapur, B. M., Allgeier, H., and Reichstein, T., *Helv. Chim. Acta*, 1967, **50**, 2147.
[5] Meister, L., Stöcklin, W., and Reichstein, T., *Helv. Chim. Acta*, 1970, **53**, 2044.
[6] Saner, A., Stöckel, K., and Reichstein, T., *Helv. Chim. Acta*, 1972, **55**, 1221.
[7] Duff, A. G., Gellert, E., and Rudzats, R., *Phytochemistry*, 1973, **12**, 2943.
[8] Gellert, E., and Summons, R. E., *Aust. J. Chem.*, 1973, **26**, 1835.

(i) Rostratine, $C_{29}H_{39}NO_8$, m.p. 259–260° from MeOH–Et_2O, $[\alpha]_D^{22}$ +49±1° (*c*, 1·0 in MeOH), R_F 0·45. Relevant and major peaks in the mass spectrum (calc. and found values agree within ±6 p.p.m.): *m/e* 529 (4·6%) M^+; 511 (0·5) $C_{29}H_{37}NO_7$; 493 (0·8) $C_{29}H_{35}NO_6$; 469 (7·5) $C_{27}H_{35}NO_6$; 454 (1·9) $C_{26}H_{32}NO_6$; 433 (1·2) $C_{27}H_{31}NO_4$; 418 (5·6) $C_{26}H_{28}NO_4$; 400 (1·9) $C_{26}H_{26}NO_3$; 373 (27) $C_{20}H_{23}NO_6$; 346 (11) $C_{21}H_{30}O_4$; 328 (5·9) $C_{21}H_{28}O_3$; 313 (4·4) $C_{18}H_{19}NO_4$; 313 (3·2) $C_{20}H_{25}O_3$; 292 (1·3) $C_{21}H_{24}O$; 289 (5·1) $C_{16}H_{19}NO_4$; 276 (4·9) $C_{15}H_{18}NO_4$; 206 (1·1) $C_{11}H_{12}NO_3$; 193 (8·9) $C_{10}H_{11}NO_3$; 175 (2·5) $C_{12}H_{15}O$; 166 (5·8) $C_{10}H_{14}O_2$; 161 (10) $C_{11}H_{13}O$; 153 (3·4) $C_9H_{13}O_2$; 150 (2·3) $C_8H_8NO_2$; 138 (8·2) $C_9H_{14}O$; 124 (100) $C_6H_6NO_2$; 123 (38) $C_6H_5NO_2$; 120 (28) C_9H_{12}; 107 (15) C_6H_5NO; 106 (55) C_6H_4NO; 105 (19) C_6H_3NO; 105 (17) C_8H_9; 91 (12) C_7H_7; 85 (35) $C_4H_5O_2$; 78 (31) C_5H_4N; 43 (54) C_2H_3O.

(ii) *Dihydrorostratine*, $C_{29}H_{41}NO_8$, m.p. 257–258° from MeOH–Et_2O, $[\alpha]_D^{22}$ +55±2° (*c*, 0·15 in MeOH), R_F 0·60. Relevant and major peaks in the mass spectrum: *m/e* 531 (0·1%) M^+; 513 (0·5); 495 (1·2); 471 (7·4); 456 (3·6); 438 (1·8); 435 (1·4); 420 (0·9); 348 (11); 330 (7·6); 289 (3·2); 276 (4·9); 206 (6·5); 167 (8·4); 150 (1·1); 124 (95); 123 (100); 106 (50); 105 (45); 78 (30); 43 (50).

Acknowledgment

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Corrigendum

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p. 24, Fig. 3, upper trace: *for* 11°C *read* −11°C.