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Adina Alkaloids : the Structure of Rubescine

W.P. Blackstock and R.T. Brown

Department of Chemistry, Manchester University, Manchester M13 9PL.

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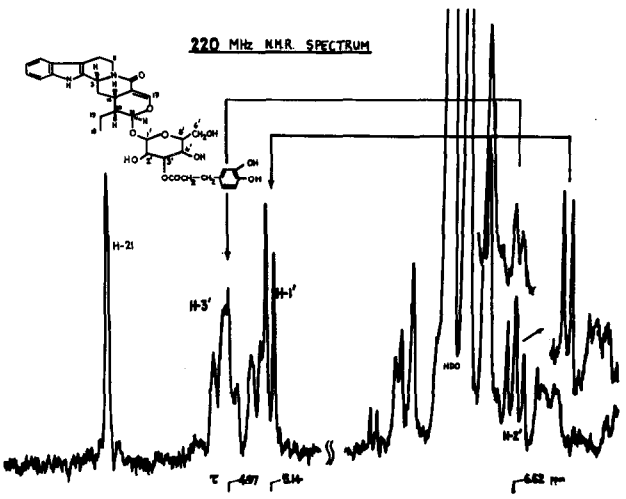
Continued fractionation of the polar constituents of Adina rubescens has yielded, in addition to those already reported[1,2], a new indolic glucoside, rubescine, for which structure (1a) is proposed on the basis of chemical and spectroscopic evidence outlined below.

A combination of gel-permeation and partition chromatography gave rubescine as a yellow amorphous powder, $[\alpha]_D^{25}$ - 121° (C=0.90, MeOH), $\lambda_{max.}^{MeOH}$ (log ϵ): 226(4.69), 275(4.25), 283(4.30), 289(4.31) and 328(4.29) nm., for which the formula $C_{35}H_{36}N_2O_{11}$ was deduced by mass measurement of its pentatrimethylsilyl ether (1e). Brief methylation with diazomethane gave a dimethyl derivative (1b), $C_{37}H_{40}N_2O_{11}$, which formed a tristrimethylsilyl derivative (1d). Catalytic hydrogenation of rubescine resulted in the formation of a tetrahydroderivative (2a), $C_{35}H_{40}N_2O_{11}$, $[\alpha]_D^{25}$ - 79° (C=1.41, MeOH), $\lambda_{max.}^{MeOH}$ (log ϵ): 227(4.55), 273(4.09), 283 (4.06) and 289(3.95) nm; C.D. (C=0.01, MeOH) $[\theta]_{267}^{o} = -31 \times 10^3$ (shoulder), $[\theta]_{292}^{o} = -7.3 \times 10^3$ (inflection). Acetylation of rubescine afforded a crystalline pentaacetate (1c), $C_{45}H_{46}N_2O_{16}$, m.p. 158-161°, $[\alpha]_D^{25}$ - 98° (C=0.15, MeOH), $\lambda_{max.}^{MeOH}$ (log ϵ): 226(4.55), 279 (4.27), 289(4.20) nm., C.D. (C=0.012, MeOH, $[\theta]_{270}^{o} = -25 \times 10^3$, $[\theta]_{292}^{o} = -9 \times 10^3$.

Pertinent features in the n.m.r. spectrum of the acetate derivative, in addition to three acetate signals at τ 7.89, 7.99 and 8.01, were two phenolic acetates appearing as a singlet at τ 7.69, and a pair of one-proton doublets (J=16 Hz) at τ 2.48 and 3.72; dimethylrubescine showed methoxyl signals at τ 6.20 and 6.23, and a similar pair of doublets. This latter feature together with the u.v. and chemical behaviour suggested the presence of a trans-dihydroxy cinnamate ester in addition to indole and β-alkoxyacrylamide chromophores.

Examination of the mass spectra of rubescine and its derivatives indicated the presence of a hexose moiety esterified by a cinnamate group. For instance, rubescine pentaacetate



FIGURE

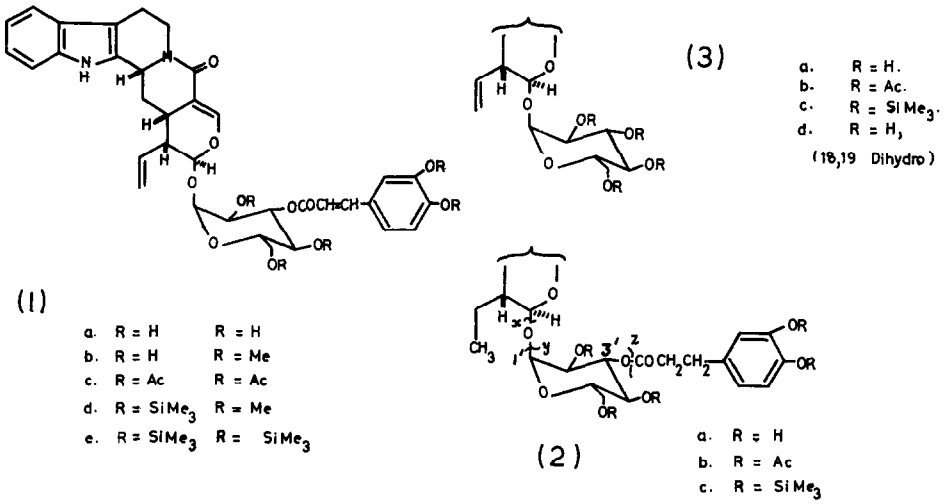


TABLE - Significant ions in mass spectra of lactam glucosides and derivatives

Structure	1b	1c	1d	1e	2b	2c	3b	3c
Ions due to M^+	688	—	904	1020	—	1024	666	786
cleavage y:								
sugar fragment	353	535	569	685	537	687	331	451
indole fragment	335	335	335	335	337	337	335	335
cleavage x:								
indole fragment	319	319	319	319	321	321	319	319
cleavage z:								
cinnamate fragment	191	247	191	307	249	309	—	—

had significant peaks at m/e 247 and 535, the former attributable to a cinnamate ion $\overset{+}{O}C\text{-}CH{=}CH\text{-}C_6H_3(OAc)_2$, and the latter to a cinnamoylated sugar triacetate fragment formed by cleavage y; these assignments were confirmed by mass measurements and by the presence of appropriate ions in the mass spectra of the derivatives (see Table).

Zemplen deacylation of rubescine gave a quantitative conversion to the known vincoside lactam (3a),[1] and an ester which was identified as methyl 3,4-dihydroxycinnamate (caffeate) from its u.v., i.r., and mass spectra, and by comparison of its dimethyl ether with an authentic sample. A similar deacylation to vincoside lactam was observed on prolonged treatment (> 30 mins.) of rubescine with diazomethane. Hence it was apparent that rubescine is vincoside lactam esterified on the glucose moiety by caffeic acid.

The location of the position of acylation on the glucose residue by traditional methods (permethylation or periodate titration) is limited by the relatively large amounts of material required, and moreover, acyl migration can lead to erroneous conclusions.[3,4] Several attempts have been made to use n.m.r. spectroscopy to circumvent this problem, either on the free glucoside[4,5,6] or on the corresponding acetates.[7] The former procedure, however, relies on the number of protons shifted downfield by acylation into the region τ 4.0 to 6.0, and while able to distinguish between substitution and C-6' and C-2', it is unable to differentiate between C2', C3' and C4'. The latter procedure depends on the assignment of acetate signals to specific positions around the glucose ring in the fully acetylated glycoside, and requires a wide range of model compounds. It is open to the criticism that the chemical shift differences are small and that changes of the same order of magnitude could occur when an acetate is replaced by a cinnamate group. Significantly, although all the acetate signals could be distinguished in both rubescine pentaacetate (1c) and vincoside lactam tetraacetate (3b), no conclusions as to the position of the caffeoyl substituent in the former could be made as the positions of <u>all</u> the acetate signals differed from those in the latter.

Catalytic hydrogenation of rubescine to the tetrahydro derivative (2a) removed the olefinic proton absorptions and gave a simplified n.m.r. spectrum between τ 4 and 6, which when compared with the corresponding region for dihydrovincoside lactam (3d) showed the

presence of an additional one proton multiplet, thus excluding acylation at C-6'. At 220 MHz.[8], the enhanced chemical shift differences allowed several additional features in the tetrahydrorubescine spectrum (see Figure) to be observed, and in consequence the complete structure to be established. A one-proton doublet at τ 5.14 (J=7.5 Hz) was attributed to the C-1' anomeric hydrogen with a trans-diaxial coupling, by analogy with other β-glucosides[9]; a one-proton triplet at τ 6.62 (J=7.5 Hz) was assigned to H-2' since irradiation of H-1' caused its collapse to a doublet (J=7 Hz). Furthermore, irradiation of a partially obscured signal at τ 4.97 also collapsed the H-2' to a doublet (J=7.5 Hz). Conversely, irradiation of the H-2' triplet effected decoupling of only the τ 4.97 and 5.14 signals as expected. The absorption at τ 4.97 was therefore assigned to H-3', shifted to lower field by acylation. Since the absolute stereochemistry of vincoside lactam has already been established,[1] the complete structure for rubescine is (1a).

References

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