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ISOLATION AND STRUCTURE OF ROSICINE FROM *CATHARANTHUS ROSEUS*

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Abstract — An investigation of the alkaloidal constituents of the leaves of *Catharanthus roseus* has led to the isolation of a new alkaloid, "rosicine", to which structure (1) has been assigned on the basis of spectroscopic studies. Two other alkaloids isolated have been identified as 14,15-dehydroepivincadine and 19-hydroxytabersonine.
Key Words — 2D-NMR, alkaloid, *Catharanthus roseus*.

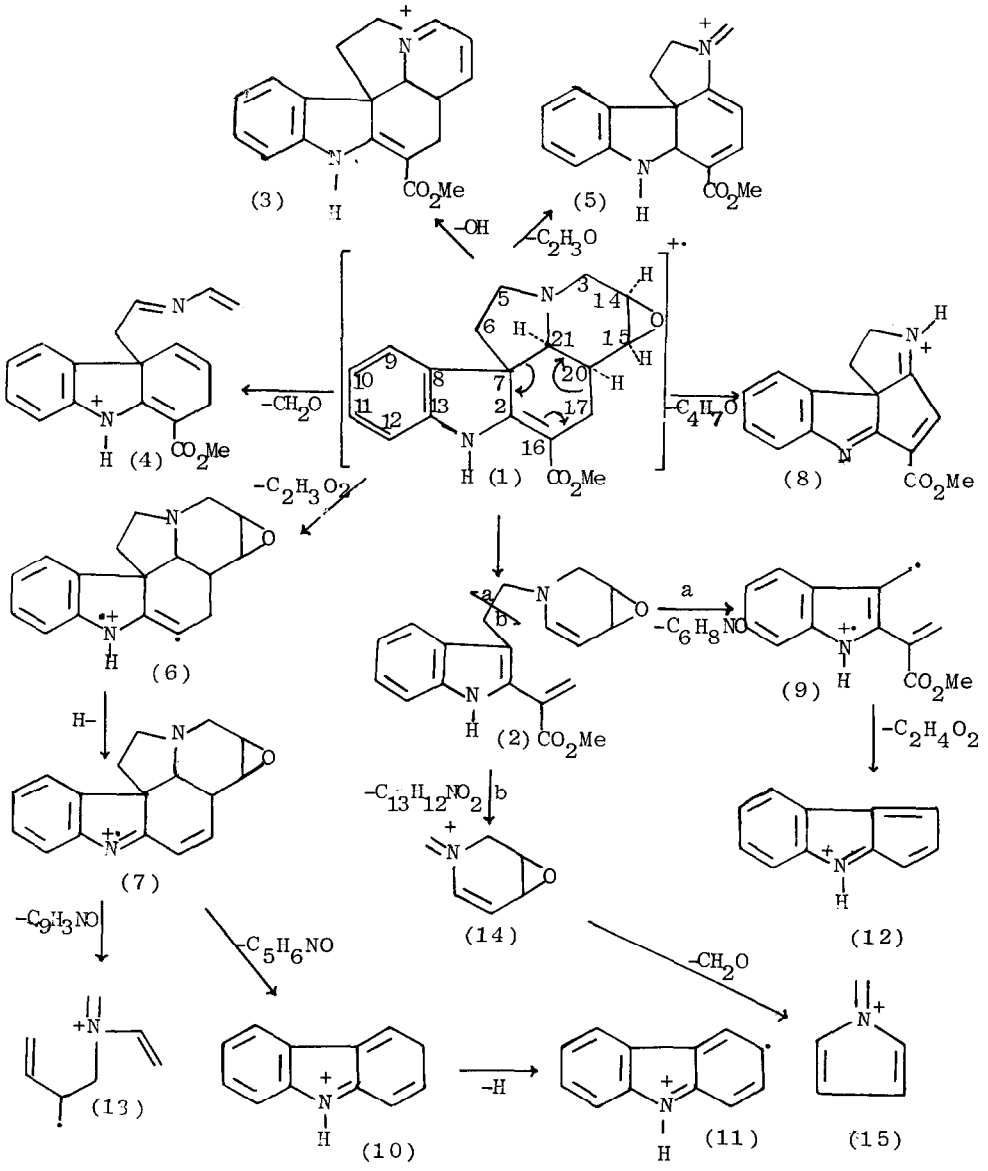
Catharanthus roseus, locally known as "sada-bahar", occurs abundantly in Pakistan. It derives its fame because of the presence in it of two highly active anti-tumour alkaloids, vinblastine and vincristine, which are used in medicine for the treatment of Hodgkin's disease, acute leukaemia in children and a number of solid tumours.[1] We have recently reported the isolation of a new alkaloid, 16-epi-19-S-vindolinine, from the leaves of this plant.[2] We now report the isolation of another new alkaloid, "rosicine" for which structure (I) is proposed on the basis of spectral studies.

The crude alkaloids obtained from the alcoholic extracts of the air-dried leaves of the plant were first extracted with aqueous phosphate buffer solution (pH-3). Selective precipitation was then carried out by the addition of petroleum-ether (40-60°) to the chloroform solution. The precipitated alkaloids were removed by filtration and the filtrate extracted with aqueous phosphate buffer solution (pH-2). The aqueous layer was washed with $CHCl_3$, basified with ammonia to pH-10 and again extracted with chloroform to afford an alkaloidal fraction which was chromatographed on a silica gel column (70-230 mesh). Successive elution with increasing polarities of mixtures of petroleum ether (40-60°) and ethyl acetate afforded a number of alkaloidal fractions, the rosicine-containing fraction being eluted with 15% ethyl acetate-85% petroleum-ether (40-60°). Further purification was carried out by preparative t.l.c. on alumina plates in 98% hexane-2% EtOAc containing a few drops of ammonia. The pure alkaloid was thus obtained as a pale yellow gum, $[\alpha]_D$ = + 188.67 ($CHCl_3$).

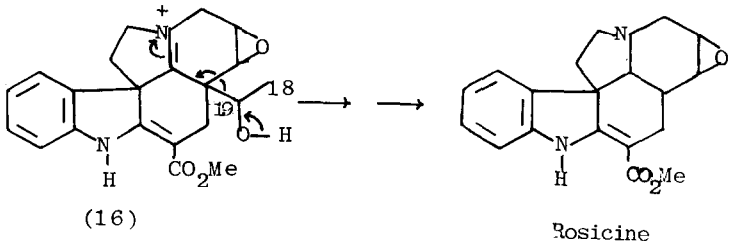
The i.r. spectrum showed a strong absorption at 1670 cm^{-1} indicating the presence of an amide or a conjugated ester group. The u.v. spectrum was characteristic of an anilinoacrylate system, exhibiting absorption maxima at 203 nm (log ε 3.64), 223 nm (log ε 3.62), 295 nm (log ε 3.60) and 325 nm (log ε 3.64)

and minima at 214 nm (log ε 3.61), 257 nm (log ε 3.44) and 304 nm (log ε 3.78).The mass spectrum afforded the molecular ion at m/z 324.1467 consistent with the formula $C_{19}H_{20}N_2O_3$ (calcd: 324.1473) indicating the presence of eleven double bond equivalents. The mass spectrum showed intense peaks at m/z 214.0862 and 110.0603 often encountered in aspidosperma-type alkaloids bearing an anilino-acrylate skeletal system.[3,4] The fragmentation pattern (scheme I) was confirmed by linked scan measurements. The typical primary fragmentation of the α-methylene indoline system was evident in the form of a retro Diels-Alder reaction according to pathways (a) or (b). Fragmentation path (a) gives the characteristic base peak at m/z 214.0862 attributed to ion (9) whereas fragmentation path (b) results in formation of the oxygen-bearing piperidine fragment at m/z 110.0606 (C_6H_8NO). Seven of the eleven double bond equivalents were accounted for by the presence of the anilinoacrylate system. The spectral data obtained did not indicate the presence of ketone or additional olefinic functionalities and it was apparent from the mass spectrum that the third oxygen was linked to the piperidine ring as an ether or in the form of an epoxide.

An analysis of the H-NMR spectrum (250 MHz, $CDCl_3$) of rosicine was undertaken and the assignments were confirmed by two dimensional H/H correlated spectrum. A three-proton singlet at δ 3.78 was assigned to the ester methyl group. The absence of any other methyl signal indicated that the ethyl or substituted ethyl side chain was absent in rosicine. A double-doublet at δ 3.50 was assigned to the C-3 β proton which showed coupling with the C-14 proton as well as with the C-3 α proton ($J_{3\beta,3\alpha}$=13Hz, $J_{3\beta,14}$ =5.2Hz). The C-3 α proton gave a doublet at δ 2.97 ($J_{3\alpha,3\beta}$=13Hz). The C-5 α proton adjacent to the nitrogen gave a multiplet at δ 2.50 showing coupling both with the β proton present on the same carbon as well as with the C-6 α proton ($J_{5\alpha,5\beta}$=8.3Hz, $J_{5\alpha,6\alpha}$=4.2Hz). The signal for the C-5 β proton resonated as a double-doublet centred at δ 2.88 being coupled with the C-5 α proton and the C-6 β proton ($J_{5\beta,5\alpha}$=8.3Hz, $J_{5\beta,6\beta}$=6.3Hz). The C-6 α proton resonated at δ 1.74 as a double-doublet ($J_{6\alpha,6\beta}$=11.6Hz, $J_{6\alpha,5\alpha}$=4.2Hz). The C-6 β proton resonated at δ 1.87 as a double-doublet being coupled with the C-5 β proton and the C-6 α proton ($J_{6\beta,6\alpha}$=11.6Hz, $J_{6\beta,5\beta}$=6.3Hz). A low field double-doublet at δ 3.38 is assigned to the C-14 proton. Two dimensional H/H correlated NMR confirmed its coupling with the C-3 β proton and with the C-15 proton, ($J_{14,3\beta}$=5.2Hz, $J_{14,15}$= 3.8Hz). Another low field one-proton doublet at δ 3.17 is assigned to the C-15 proton ($J_{15,14}$=3.8Hz). The C-17 α-H exhibited a double-doublet at δ 2.38 ($J_{17\alpha,17\beta}$=14.5Hz, $J_{17\alpha,20}$=12Hz). The corresponding β proton at C-17 gave a double-doublet at δ 2.60 ($J_{17\beta,17\alpha}$=14.5Hz, $J_{17\beta,20}$=2Hz). A multiplet at δ 1.89 was assigned to the C-20 proton ($J_{20,17\beta}$=2Hz, $J_{20,21}$=3Hz, $J_{20,17\alpha}$=12Hz). The aromatic protons resonated as complex multiplets in the range of δ 6.84 - δ 7.28. These coupling data were consistent with structure (I) assigned to rosicine.



Scheme - I



Scheme - 2

The C-13 NMR assignments were made after comparison with the C-13 NMR spectra of other Aspidosperma-type alkaloids.[5] Gated spin echo measurements revealed the presence of four methylenes which resonated at δ 21.37, δ 44.76, δ 50.21 and δ 50.61 and were assigned to C-17, C-6, C-3 and C-5 respectively. The rather upfield value of C-17 in comparison to tabersonine is attributed to the presence of an epoxy group at C-14/C-15 due to a γ-effect. This suggests that the epoxide is oriented *cis* to C-17. The upfield displacement of this signal by 3 ppm in comparison to the corresponding signal in hazuntinine is consistent with the absence of the ethyl side chain at C-20; this was further indicated by comparing the calculated chemical shift values for C-17 with and without the ethyl side chain on C-20. The chemical shift of C-6 at δ 44.76 was consistent with the observed value of this carbon between δ 44- δ 46 ppm throughout the Aspidosperma alkaloidal series.[5]

Gated spin echo measurements showed the presence of eight methine carbon atoms which resonated at δ 38.2, δ 52.4, δ 55.4, δ 67.2, δ 109.3, δ 120.6, δ 121.5 and δ 127.8 and were assigned to C_{20}, C_{15}, C_{14}, C_{21}, C_{12}, C_{10}, C_9 and C_{11} respectively. It could be shown by gated spin echo measurements that the oxygen bearing carbon atoms were methines and not methylenes. The assignments at δ 55.4 and δ 52.4 for C-14 and C-15 were in agreement with these data. Moreover the chemical shift values for C-14 and C-15 were close to those found in hazuntinine. Six quaternary carbons were found to resonate at δ 55.26, δ 95.2, δ 136.9, δ 143.4, δ 166.59 and δ 168.3 and were assigned to C-7, C-16, C-8, C-13, C-2 and the ester C=O respectively. The spectrum showed one downfield methyl signal at δ 51.007 which is due to the OMe of the ester group. Interestingly, the C-13 NMR of rosicine did not show any upfield C-18 methyl signal characteristically found in Aspidosperma alkaloids in the range δ 7.9-7.3 ppm nor was the methylene of the ethyl group present. These data are consistent with structure (I) of rosicine.

Rosicine is one of the rare indole alkaloids which lack the ethyl group present in Aspidosperma alkaloids such as tabersonine. It could arise in the plant by a fragmentation of a hydroxyethyl precursor (16) as shown in scheme-2. Compounds bearing this skeletal system have recently been synthesised.[6]

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