Leurosinone: A New Binary Indole Alkaloid from *Catharanthus roseus*

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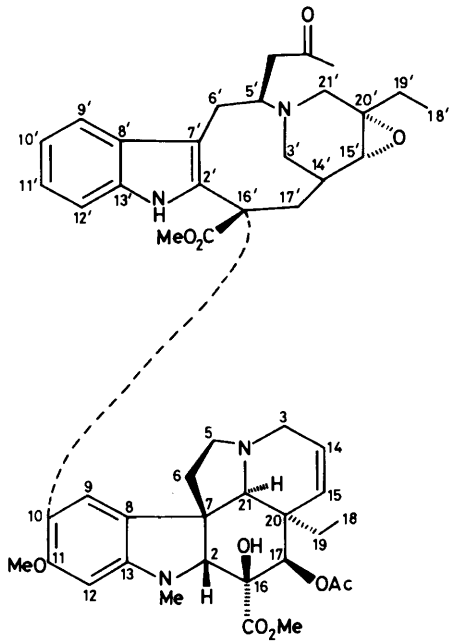
A new binary indole alkaloid, leurosinone, has been isolated from the leaves of *Catharanthus roseus*, and has been assigned structure (**1**) on the basis of spectral studies.

Catharanthus roseus (L) G. Don[1] (Apocynaceae) is widely distributed throughout Pakistan. A number of indole and binary indole alkaloids have previously been reported from this plant of which vinblastine (VLB) and vincristine play an important part in human chemotherapeutic management.[2,3] During studies on this plant, we have isolated a new binary alkaloid, leurosinone, to which structure (**1**) has been



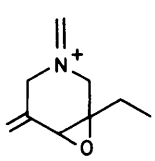
(**1**)

assigned on the basis of n.m.r. studies including homo-decoupling experiments. Its stereochemistry has been determined by a series of n.O.e. difference measurements and ^{13}C n.m.r. assignments made by DEPT pulse sequence and GASPE experiments.

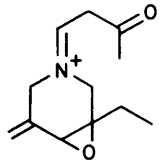
Results and Discussion

The u.v. spectrum of compound (**1**) indicated the presence of both indole and dihydroindole chromophores with $\lambda_{max.}$ (MeOH) 214, 260, and 296 nm, which typifies vinblastine.[4,5] The i.r. spectrum showed absorptions at 3 460 (NH and OH), 1 730 cm^{-1} (saturated ester), and also an additional carbonyl at 1 710 cm^{-1}.

The high resolution mass spectrum showed the molecular ion at *m/z* 864.4349 ($C_{49}H_{60}N_4O_{10}$). The M^+ was further confirmed by FAB and FD mass spectrometry. Linked scan measurements on the molecular ion showed that the ion at *m/z* 807.3977 ($C_{46}H_{55}N_4O_9$) arose directly from it, indicating the loss of 57 a.m.u. (*i.e.* – CH_2Ac or its equivalent) from the molecular ion. An examination of the fragmentation pathway of the ion at *m/z* 807 by linked scan measurements showed that the following ions arose directly from it: *m/z* 766, 749, 648, 455, and 351. The overall fragmentation pattern was very similar to that of leurosine.[6]



(**I**)



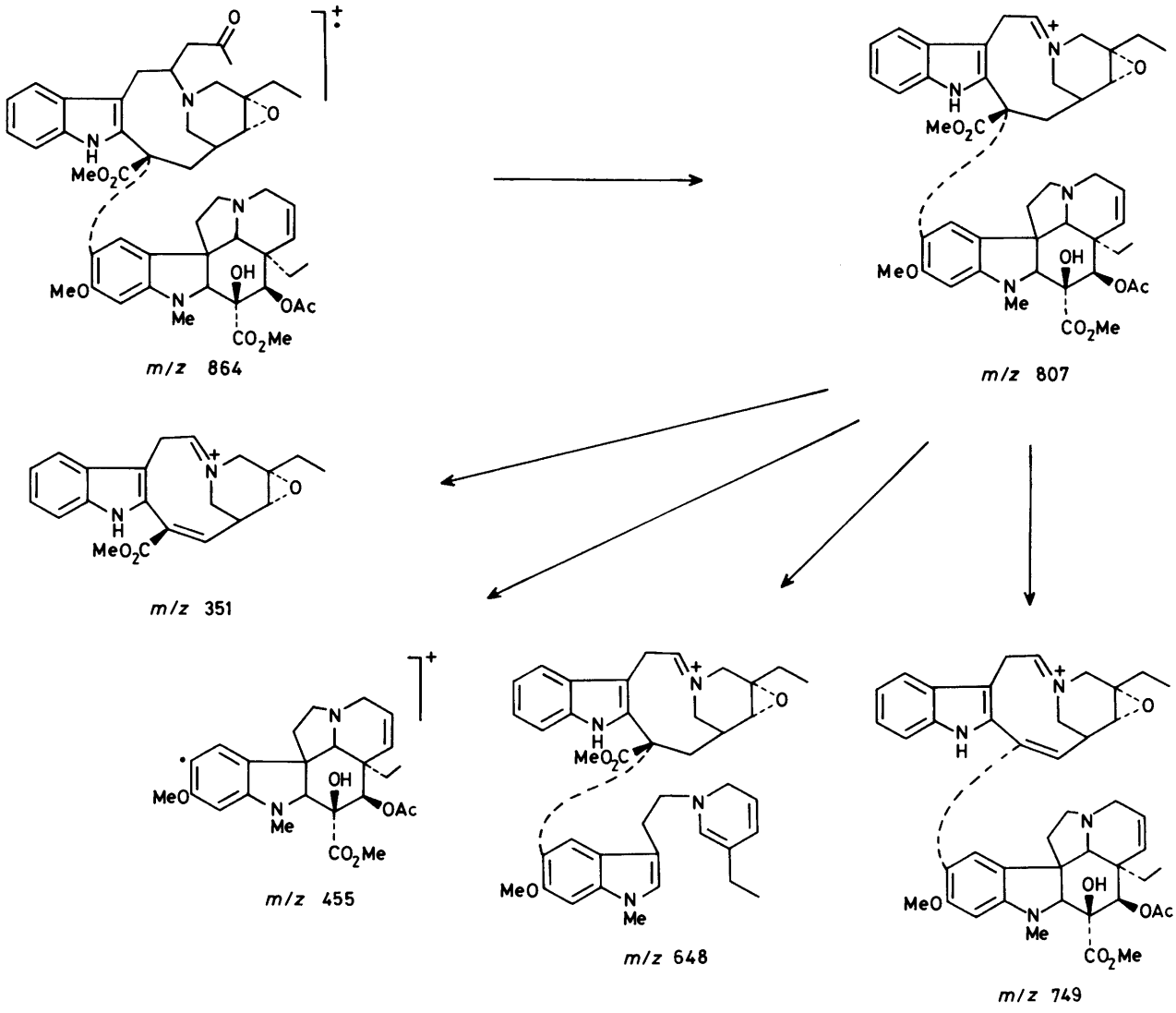
(**II**)

The ions at *m/z* 152.1068 ($C_9H_{14}NO$) [ion (**I**)] and at *m/z* 208.1327 ($C_{12}H_{18}NO_2$) [ion (**II**)] are consistent with the presence of an epoxide function in the piperidine ring. Moreover, ion (**II**) shows that the CH_2Ac unit is attached near the piperidine unit. The possibility of the CH_2Ac group being present in the vindoline half of the molecule was eliminated as the normal fragmentation of the vindoline[6–8] moiety with ions at *m/z* 296, 282, 188, 174, 135, 122, and 107 was observed. The overall fragmentation pattern of leurosinone (**1**) is shown in Schemes 1 and 2.

The ^1H n.m.r. spectrum of leurosinone (**1**) was consistent with the binary nature of the molecule. Analysis of the data showed that a vindoline moiety substituted at the 10-position was present. Two three-proton singlets at δ 3.78 and 2.12 were assigned to the methyl groups of the 16-methoxycarbonyl and 17-acetoxy groups, respectively. The 11-OMe group on the aromatic ring resonated as a three-proton singlet at δ 3.80 whereas the NMe protons appeared as another three-proton singlet at δ 2.70.[9,10] The methylene protons of the 20-ethyl group appeared as two multiplets centred at δ 1.33 (19α-H) and 1.85 (19β-H) indicating their non-equivalence due to their prochiral nature.[11] The 18-methyl protons appeared as a triplet at δ 0.79 ($J_{18,19\alpha} = J_{18,19\beta} = 7.2$ Hz). Irradiation of the methyl protons at δ 0.79 resulted in both the 19-methylene protons signals at δ 1.33 and 1.85 collapsing into doublets, each showing geminal coupling only ($J_{19\alpha,19\beta}$ 13.6 Hz). Reciprocal decoupling effects were also observed when the 19-methylene protons were irradiated.

A doublet at δ 5.28 ($J_{15,14}$ 10.4 Hz) was assigned to the olefinic proton at C-15.[9–10] The other olefinic proton at C-14 resonated at δ 5.84 and was coupled with the 3α- and 3β-methylene protons ($J_{14,3\alpha} = J_{14,3\beta} = 3.6$ Hz). The 3α-H resonated as a multiplet at δ 3.34 whereas the 3β-H appeared at δ 3.39 ($J_{3\alpha,3\beta} = 12.0$ Hz). Irradiation at δ 3.34 resulted in the multiplet of the 14-H at δ 5.84 collapsing into a quartet ($J_{15,14}$ 10.4, $J_{14,3\beta}$ 3.6 Hz). Irradiation of 14-H at δ 5.84 led to the collapse of the multiplets at δ 3.39 and 3.34 into simple doublets, showing only geminal coupling between 3α- and 3β-protons.

One-proton singlets were observed at δ 6.10 (12-H) and 6.59 (9-H), typical of shielded aromatic protons in a *para* relationship. This established that the point of attachment of the indole moiety was at C-10, analogous to vinblastine.[9,10,12] The



Scheme 1.

splitting patterns of the C-18 and C-18′ protons indicated that the two ethyl side-chains were unsubstituted.

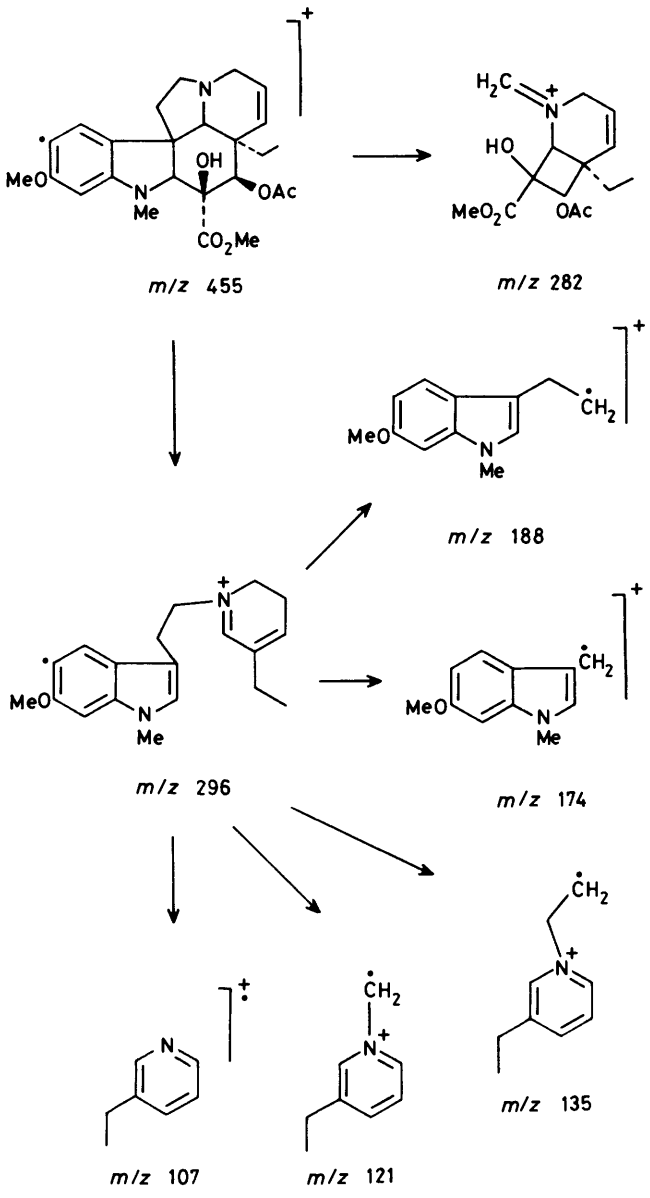
The principal ^{1}H n.m.r. resonances of the indole moiety were observed at δ 0.96 (18′-Me), 3.60 (16′-CO_2Me), 7.07 (10′-H), 7.10 (11′-H), 7.15 (12′-H), and 7.44 (9′-H),[13] with an exchangeable indole NH appearing at δ 7.96. The methylene protons of the 20′-ethyl group appeared as two multiplets centred at δ 1.30 (19′α-H) and 1.79 (19′β-H), indicating their non-equivalence. The 18′-methyl protons appeared as a triplet at δ 0.96 ($J_{18',19'\alpha} = J_{18',19'\beta} = 7.4$ Hz). The presence of a 15′,20′-epoxide function was established by the n.m.r. signal at δ 3.29 (doublet, $J_{14',15'}$ 4.1 Hz), consistent with the presence of an epoxymethine 15′-proton coupled with the 14′-proton.

The two most striking differences on comparison of the ^{1}H n.m.r. spectrum of leurosinone (**1**) with that of leurosine were the presence of an additional 3 H singlet at δ 2.09 assigned to the methyl of an acetyl group and the absence of the doublet for the 5′β-proton at δ 3.67.[14] The absence of this doublet suggested that the CH_2Ac group was attached at this position in a β-configuration in leurosinone (**1**).

A series of n.O.e. difference measurements were carried out to ascertain the position and stereochemistry of the CH_2Ac in leurosinone (**1**). The n.O.e. results are presented in Table 1 and confirm the structure of leurosinone as (**1**).

Support for the structure (**1**) was provided by the broad band 'Gated spin echo,[15] (GASPE) and 'Distortionless enhancement by polarisation transfer'[16] (DEPT) ^{13}C n.m.r. experiments. The ^{13}C n.m.r. data of leurosinone (**1**) are presented in Table 2. The presence of a vindoline moiety in the binary alkaloid was apparent from the very close correspondence in chemical shifts of the carbon atoms in the vindoline moiety of leurosinone with those of leurosine and vinblastine.[17,18] The ^{13}C chemical shifts also served to establish the expected linkage of the indole moiety at C-10 of vindoline.

In the indole moiety, the chemical shifts of both the aromatic and aliphatic carbons corresponded closely with those of leurosine.[17] Also, the methoxycarbonyl group afforded characteristic resonances at δ 52.38 and 174.30. Moreover, the presence of an epoxide function could be deduced from the signal at δ 62.25 which is consistent with a quaternary carbon of an oxirane system and assignable to C-20′; the other carbon of the oxirane system resonated at δ 63.62 and was assigned to C-15′. The presence of an epoxide unit at C-15′–C-20′ in leurosinone (**1**) was substantiated by the γ-effect, due to the



Scheme 2.

epoxide, which was exerted on the 18′-methyl in comparison with that on the corresponding carbon in vinblastine. A 2.5 p.p.m. reduction in the γ-effect on C-18′ in leurosinone (**1**) (δ 6.7 in VLB, δ 9.2 in leurosinone) parallels the effect observed in leurosine[17] and confirms the replacement of a 20′-hydroxy group by a 20′-ether linkage.

The most striking difference in the ^{13}C n.m.r. of leurosinone (**1**) in comparison with that of leurosine was the the presence of an additional carbonyl carbon resonance at δ 209.40, a methylene carbon at δ 38.77, a low-field value of the methyl carbon at δ 31.66, and a methine carbon at δ 56.28. These data suggested the presence of a CH_2Ac group at C-5′. The location of the substituent at C-5′ rather than at any other site was indicated by the low-field value of the methine carbon, the chemical shift of which (δ 56.28) was in agreement with it being adjacent to a nitrogen atom.

Leurosinone (**1**) probably arises from leurosine by oxidation at the 5′-N bond to the corresponding immonium species, which is then trapped by a molecule of acetone. Acetone has previously been shown to be involved in the biosynthesis of other complex natural products.[19] Thus, to establish that leurosinone (**1**) is not formed during isolation, the extraction was carried out with the rigorous exclusion of acetone, and leurosinone (**1**) was detectable both in the fresh chloroform extracts as well as in the purified fractions.

Table 1. Connectivities established by n.O.e. difference spectra

Proton irradiated	Chemical shifts (δ)	n.O.e. connectivity
NMe	2.70	16-CO_2Me, 2-H, 17-H
2-H	3.72	NMe, 6β-H, 12-H
17-H	5.46	19α-H, 19β-H
15-H	5.28	18-Me, 19α-H, 14-H
14-H	5.84	15-H, 3α-H, 3β-H
3α-H	3.34	14-H, 3β-H, 21-H
3β-H	3.39	3α-H, 14-H
5α-H	2.38	3α-H, 5β-H, 21-H
5β-H	2.80	5α-H
6α-H	2.55	6β-H, 9-H, 21-H
6β-H	2.06	6α-H, 2-H
9-H	6.59	N-H, 6α-H, 21-H, 3′β-H
12-H	6.10	2-H, 11-OMe, NMe
21-H	2.61	18-Me, 5α-H, 6α-H, 9-H, 3α-H
17′α-H	2.34	17′β-H, 14′-H, 15′-H, 5′a-H
17′β-H	3.78	6′β-H, 17′α-H, 15′-H
14′-H	1.23	17′α-H, 15′-H, 3′α-H, 3′β-H
15′-H	3.29	17′α-H, 17′β-H, 14′-H, 21′β-H
21′α-H	2.77	21′β-H, 18′-Me
21′β-H	2.40	15′-H, 21′α-H, 19′β-H
5′α-H	3.22	6′α-H, 17′α-H
6′α-H	3.02	5′α-H, 6′β-H, 9′-H
6′β-H	3.18	6′α-H, 17′β-H, 5′-CH_2Ac
9′-H	7.44	6′α-H, 10′-H, 5′-CH_2Ac
Indole NH	7.96	18-Me, 9-H, 12′-H, 16′-CO_2Me
5′-CH_2*Ac*	2.09	3′β-H, 19′β-H
5′-CH_2Ac	2.94	6′β-H, 9′-H

Table 2. ^{13}C N.m.r. spectrum of leurosinone

Vindoline unit		Indole unit	
Carbon	Chemical shift (δ)	Carbon	Chemical shift (δ)
2	83.46	2′	131.18
3	50.51[a]	3′	43.30
5	50.68[a]	5′	56.28
6	44.63	6′	29.53
7	53.29	7′	116.87
8	123.19	8′	129.23
9	123.57	9′	118.41
10	120.98	10′	122.40
11	158.13	11′	119.01
12	94.36	12′	110.42
13	153.50	13′	134.92
14	124.44	14′	32.95
15	130.07	15′	63.62
16	79.67	16′	55.35
17	76.46	17′	28.87
18	8.44	18′	9.22
19	30.89	19′	28.24
20	42.85	20′	62.25
21	66.11	21′	54.01
CO_2Me	170.83	CO_2Me	174.30
CO_2*Me*	52.17	CO_2*Me*	52.38
NMe	38.38	5′-CH_2Ac	38.77
OCOMe	171.69	5′-CH_2COMe	209.40
OCO*Me*	21.10	5′-CH_2CO*Me*	31.66
ArO*Me*	55.88		

[a] Assignments are interchangeable.

Experimental

M.p.s were obtained on a Gallenkamp apparatus. ^1H (300.13 MHz) and ^{13}C (75.47 MHz) N.m.r. spectra were recorded in $CDCl_3$ on a Bruker AM-300 FT spectrometer. Chemical shifts are in p.p.m. (δ) from $SiMe_4$ as internal standard. The i.r. spectra were run on a JASCO-IR A-1 spectrophotometer in $CHCl_3$, and u.v. spectra on a Shimadzu U.V. 240 spectrophotometer in MeOH. Optical rotations were measured on a Schmidt and Haensch polartronic-D electronic polarimeter in $CHCl_3$. Mass spectra were obtained with Finnigan MAT 112 and Finnigan MAT 312 double-focussing mass spectrometers.

Isolation of Leurosinone (**1**).—Air-dried leaves (20 kg) of *Catharanthus roseus* (L) G. Don were powdered and extracted with chloroform. The chloroform extracts were combined, filtered, and evaporated under reduced pressure to a gum. The gummy material (200 g) was partially dissolved in 2% aqueous tartaric acid (1 l). After the undissolved material was filtered off, the aqueous solution (pH 2.9) was extracted with chloroform (3 × 2 l). The organic extracts were removed, and the pH of the aqueous acidic phase was adjusted with aqueous ammonia (28%) to pH 5.0. Further extraction with chloroform (3 × 2 l) afforded an alkaloidal fraction (60 g). This was chromatographed on a column (60 mm diameter) packed with silica gel PF-254 (2 kg). Elution was carried out with increasing polarities of light petroleum–chloroform, chloroform, and chloroform–methanol. The eluates obtained from chloroform–methanol (9:1) afforded an alkaloidal mixture which was concentrated and separated by preparative t.l.c. on precoated silica gel plates (layer thickness 0.2 mm) in ethyl acetate–ethanol (8:2). A faster moving band was scraped off and elution with methanol afforded a pure amorphous alkaloid (**1**) (30 mg) (R_F 0.34); $[\alpha]_D^{24}$ +86° (*c* 0.02 in $CHCl_3$); $\lambda_{max.}$(MeOH) 214, 260, and 296 nm; $\lambda_{min.}$ 246 and 284 nm; $\nu_{max.}$($CHCl_3$) 3 460 (NH, OH), 2 950 (CH), 1 730 (ester C=O), 1 710 (C=O), 1 615, (C=C), 1 040 (CO), and 745 cm^{-1} (ArCH); *m/z* 864 (M^+, 7%), 807 (93), 749 (8), 706 (24), 648 (4), 351 (7), 296 (2), 282 (3), 222 (6), 208 (14), 188 (11), 174 (4), 152 (12), 135 (61), 122 (47), 121 (35), and 107 (29); δ_H($CDCl_3$, 300.13 MHz) 0.79 (3 H, t, $J_{18,19}$ 7.2 Hz, 18-Me), 0.96 (3 H, t, $J_{18',19'}$ 7.4 Hz, 18′-Me), 2.09 (3 H, s, 5′-CH_2Ac), 2.12 (3 H, s, 17-OAc), 2.70 (3 H, s, NMe), 3.60 (3 H, s, 16′-CO_2Me), 3.72 (1 H, s, 2-H), 3.78 (3 H, s, 16-CO_2Me), 3.80 (3 H, s, 11-OMe), 5.28 (1 H, d, $J_{15,14}$ 10.4 Hz, 15-H), 5.46 (1 H, s, 17-H), 5.84 (1 H, m, 14-H), 6.10 (1 H, s, 12-H), 6.59 (1 H, s, 9-H), 7.08–7.16 (3 H, m, 10′-, 11′-, and 12′-H), and 7.44 (1 H, d, *J* 7.5 Hz, 9′-H); δ_C($CDCl_3$, 75.47 MHz) data are reported in Table 2.

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Received 22*nd October* 1986; *Paper* 6/2059