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16-EPI-19-*S*-VINDOLININE, AN INDOLINE ALKALOID FROM *CATHARANTHUS ROSEUS*

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Key Word Index—*Catharanthus roseus*; Apocynaceae; leaves; indole alkaloids; ^{13}C NMR; 16-epi-19-*S*-vindolinine.

Abstract—Studies on the alkaloids of *Catharanthus roseus* have resulted in the isolation of a new alkaloid, to which the structure of 16-epi-19-*S*-vindolinine has been assigned.

INTRODUCTION

In continuation of our isolation and synthetic studies on the alkaloids of *Catharanthus roseus* [1–15] we have isolated a new alkaloid from the leaves of this plant by a combination of selective extraction, precipitation and crystallization.

RESULTS AND DISCUSSION

The alkaloids were isolated from the leaves of *C. roseus* by usual procedures in a yield of 0.6% of the weight of air-dried material. A new alkaloid, mp 200°, $[\alpha]_D + 40°$ (MeOH), was isolated by a combination of selective extraction, precipitation and crystallization, as a white crystalline solid in a yield of 0.65% by weight of the alkaloidal mixture. The substance afforded a UV spectrum which was typical of a dihydroindole system, showing absorption maxima at 212, 246 and 303 nm and minima at 276 and 226 nm. The IR spectrum showed the presence of an ester carbonyl absorption at 1730 cm^{-1}.

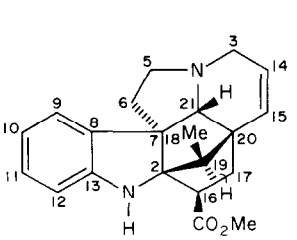
The new alkaloid afforded a mass spectrum which was very similar to that reported for vindolinine [16] and 19-epi-vindolinine [17]. A high resolution mass measurement on the M^+ showed the exact mass to be *m/z* 336.1837 in agreement with the formula $C_{21}H_{24}N_2O_2$.

The ^{13}C NMR spectrum of the new alkaloid (**1**) (broad band and off-resonance) showed interesting similarities to that reported for 19-*R*-vindolinine [18], 19-*S*-vindolinine [18] and 16-epi-19-*R*-vindolinine [18]. The ester carbonyl carbon resonated at δ173.47 whereas the methyl of the ester group resonated at δ 52.6 (quartet). The substance afforded four doublets for the tertiary aromatic carbons, and two singlets for the two quaternary aromatic carbon atoms. A characteristic singlet appeared at δ 81.36 corresponding to the quaternary carbon atom α to the indoline nitrogen. The assignments of the ^{13}C resonances are shown in Table 1 in comparison with the resonances of 19-*R*-vindolinine, 19-*S*-vindolinine and 16-epi-19-*R*-vindolinine.

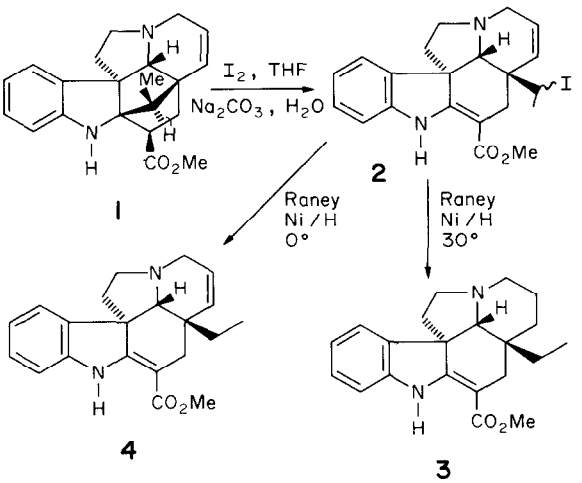
The ^1H NMR spectrum of **1** recorded at 200 MHz showed the presence of a doublet at δ 0.62 (J = 7.4 Hz) which is assigned to the C-18 methyl protons. The proton adjacent to the carbomethoxyl function resonated as a double doublet at δ 3.18 (J_1 = 12.2 Hz, J_2 = 5.8 Hz). A quartet at δ 2.26 (J = 7.4 Hz) was assigned to the C-19 proton. A double doublet at δ 6.41 was assigned to the olefinic proton at C-15, showing coupling with the vicinal olefinic proton and an allylic coupling with the C-3 proton (J_1 = 10 Hz, J_2 = 2.8 Hz). The other olefinic proton at C-14 resonated as a doublet of double doublets at δ 5.84 (J_1 = 10 Hz, J_2 = 5.2 Hz, J_3 = 1.8 Hz). The chemical shift of δ 0.62 for the methyl group is consistent with a 19-*S*-configuration as the methyl group of 19-*S*-vindolinine resonates at δ 0.57, whereas the methyl group in 19-*R*-vindolinine resonates at δ 0.95.

Direct TLC comparison with authentic samples of vindolinine and epi-vindolinine showed that the substance could just be separated from these two materials on Si gel in ethyl acetate–ethanol (3:1).

In order to confirm the structure, **1** was subjected to



1



Scheme 1.

Table 1. ^{13}C NMR spectral data of **1**, 19-*R*-vindolinine, 19-*S*-vindolinine and 16-epi-19-*R*-vindolinine

Carbon No.	Chemical shifts*				Multiplicites
	19-*R*-Vindolinine	19-*S*-Vindolinine	16-Epi-19-*R*-Vindolinine	Alkaloid (**1**)	
2	81.4	80.4	80.5	81.36	singlet
3	58.0	57.5	57.4	58.14	triplet
5	50.3	49.8	50.1	48.9	triplet
6	36.3	37.2	35.0	34.66	triplet
7	59.8	58.4	60.7	59.98	singlet
8	139.8	139.0	135.7	135.30	singlet
9	123.6	123.1	123.1	123.70	doublet
10	121.0	121.2	118.9	122.74	doublet
11	127.2	127.0	126.9	125.9	doublet
12	112.0	111.8	109.0	112.71	doublet
13	149.4	149.2	148.7	148.46	singlet
14	128.5	128.1	128.2	128.61	doublet
15	130.7	131.0	130.6	132.56	doublet
16	39.2	42.7	39.4	38.96	doublet
17	29.1	31.4	31.9	28.73	triplet
18	7.4	10.1	7.8	7.15	quartet
19	48.4	51.2	44.8	48.46	doublet
20	46.2	44.2	47.8	44.58	singlet
21	78.0	74.2	76.4	74.19	doublet
O ‖ -C-O	174.2	172.8	174.5	173.47	singlet
-O-Me	51.8	51.2	51.7	52.6	quartet

*Chemical shift values are given in δ values (ppm) from TMS. All values are for solutions of the compound in chloroform.

oxidative cleavage reaction [19, 20] with iodine–THF–water–sodium carbonate when it was converted to the iodo compound (**2**). On hydrogenolysis with Raney nickel at 30° for 2 hr, the iodo compound was transformed to (−)-vincadifformine (**3**). When the same hydrogenolysis experiment was repeated at 0° for 5 min, quantitative conversion to tabersonine (**4**) was observed. The identity of the synthetic hydrogenolysis products was established by direct chromatographic and spectroscopic comparisons with authentic samples of tabersonine and vincadifformine (Scheme 1).

The above sequence of experiments conclusively establish that the new alkaloid has the same stereochemistry at C-7, C-20 and C-21 as vindolinine. Furthermore, since it can be converted to (−)-vincadifformine, $[\alpha]_D$ −542° (EtOH), it must have the same absolute configuration at C-7, C-20 and C-21 as (−)-vincadifformine and hence belong to the same enantiomeric series. As the ^1H NMR had indicated a 19-*S*-configuration, and as **1** differed in its ^1H and ^{13}C NMR from the three known diastereoisomers of vindolinine (i.e. 19-*R*-vindolinine, 19-*S*-vindolinine, 16-epi-19-*R*-vindolinine), it is assigned to be 16-epi-19-*S*-vindolinine (**1**).

EXPERIMENTAL

MS were measured on double focussing instruments. TLC was carried out on Si gel GF-254 pre-coated plates (E. Merck).

Isolation of alkaloid **1**. Air-dried leaves of *C. roseus* (16 kg) were finely crushed with an Ultra-turrax in 32 l. EtOH. The crushed material was filtered and washed thoroughly with 7 l. EtOH. The EtOH filtrates were combined and evaporated under vacuum to a gum. The gum was acidified with 5% HCl (4 l.) and washed with $CHCl_3$ (3 l.). The ice-cold soln was then basified (700 ml, 33% NH_3 soln) and extracted with $CHCl_3$ (4 l.). The $CHCl_3$ extracts were dried (Na_2SO_4) and concd to the crude alkaloidal gum (100 g).

The alkaloids (100 g) were dissolved in 300 ml $CHCl_3$ and extracted with Pi buffer pH 3 (1 l.). The $CHCl_3$ layer was dried (Na_2SO_4), filtered and concd under vacuum to afford 50 g of alkaloid mixture. This was dissolved in 200 ml $CHCl_3$ and 400 ml hexane was added, which caused selective pption of some alkaloids. The ppts were filtered off and the filtrate again concd to a gum (30 g). The gum was dissolved in EtOAc (200 ml) and extracted with Pi buffer pH 2 (1 l.). The aq. layer was separated, extracted with $CHCl_3$ (1 l.) and concd to a gum (12.5 g). Addition of Me_2CO resulted in the crystallization of **1** which was recrystallized from EtOH as a white crystalline solid, mp 200°; $[\alpha]_D$ +40° (MeOH); IR ν_{max}^{KBr} cm^{-1}: 1730; UV λ_{max}^{MeOH} nm: 212, 246, 303; λ_{min}^{MeOH} nm: 226, 276; NMR ($CDCl_3$): δ 0.62 (3H, *d*, J = 7.4 Hz, CH–CH_3), 2.26 (1H, *q*, J = 7.4 Hz, C<u>H</u>–Me), 3.18 (1H, *dd*, J_1 = 12.2 Hz, J_2 = 5.8 Hz, H-16), 5.84 (1 H, *ddd*, J_1 = 10 Hz, J_2 = 5.2 Hz, J_3 = 1.8 Hz, H-14), 6.41 (1 H, *dd*, J_1 = 10 Hz, J_2 = 2.8 Hz, H-15), 3.76 (3 H, *s*, OCH_3); MS: *m/z* 336 $[M]^+$ (57), 230 (31), 229 (28), 216 (23), 170 (86), 135 (94), 134 (100), 122 (23), 121 (24), 120 (30), 93 (20), 77 (21); high resolution mass measurements: 336.1837 ($C_{21}H_{24}N_2O_2$ requires 336.1837), 230.1155 ($C_{14}H_{16}NO_2$ requires 230.1180), 170.0957 ($C_{12}H_{12}N$ requires 170.0969), 135.1039 ($C_9H_{13}N$ requires 135.1047), 134.0965 ($C_9H_{12}N$ requires 134.0969).

Cleavage of **1** *to* **2**. Alkaloid **1**, (200 mg 0.59 mmol) was dissolved in THF (20 ml) and I_2 (175 mg) was added. The soln was stirred for 4 hr at 30°. Then 10% Na_2CO_3 soln (5 ml) was added and the soln stirred for 1.5 hr. The soln was evaporated and the reaction mixture was partitioned between $CHCl_3$ (20 ml) and H_2O (20 ml).

The $CHCl_3$ layer was separated, dried (Na_2SO_4) and concd under vacuum to afford a gummy mass. On TLC (hexane–EtOAc, 4:1), it showed the formation of a faster running product and some unreacted starting material. This was purified by flash chromatography through a small Si column. Elution with EtOAc–petrol (1:19) afforded pure **2** (70% yield), which was identical in its spectroscopic properties with those reported in the lit. [20].

Reduction of **2** *to* **3**. Compound **2** (20 mg, 0.043 mmol) was dissolved in MeOH (2 ml) and added to a suspension of Raney Ni (30 mg) satd with H_2 in MeOH (5 ml) containing NaOH (10 mg). The soln was stirred for 2.5 hr at 30°, filtered, concd and fractionated between $CHCl_3$ (10 ml) and H_2O (10 ml). The $CHCl_3$ layer was separated, dried (Na_2SO_4) and concd to afford a gum which on TLC showed complete conversion to a slower moving substance (13 mg, 86% yield). The substance was purified by prep. TLC and chromatographic, as well as spectroscopic, comparison with an authentic sample of vincadifformine showed the two to be identical. The synthetic material afforded $[\alpha]_D$ −542° (EtOH).

Reduction of **2** *to* **4**. To 30 mg of Raney Ni suspension in MeOH (5 ml) satd with H_2 was added **2** (20 mg, 0.043 mmol) dissolved in 2 ml MeOH containing NaOH (10 mg.). The soln was stirred for 5 min at 0°, filtered, concd and fractionated between $CHCl_3$ (10 ml) and H_2O (10 ml). The $CHCl_3$ layer was separated, dried (Na_2SO_4) and concd to afford a gummy product, which on TLC showed complete conversion to a slower moving material (14 mg, 90% yield). The substance was purified by prep. TLC and chromatographic, as well as spectroscopic, comparison with an authentic sample of tabersonine showed the two to be identical.

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*This patent describes a synthesis of vinblastine identical to that subsequently published by P. Potier *et al.* (*J. Am. Chem. Soc.* **101**, 2243).

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