MeOH–H_2O (7:3) for 30 min, with rotation, at 40°. The suspension was filtered and the filtrate was evapd *in vacuo* at 30° to 50 ml and shaked with 4 × 50 ml petrol–Et_2O (3:1). The water phase was separated and extracted with 8 × 50 ml EtOAc–MeOH (97:3). The organic phase was dried, filtered and evapd at 30° (442 mg). A part from this crude extract was dissolved in 3 ml MeOH, filtered and added to the chromatographic system A. The faster eluting zones were coned to yield **3** which was purified by system B and identified by partial hydrolysis and direct comparison with an authentic compound. The later zones yielded **1** which was purified by system B (5 mg), $[\alpha]_D^{20} = -110.94$ (MeOH; *c* 0.32), $R_f = 0.41$. FABMS: *m/z* 793 $[M+Na]^+$. For ^1H NMR (in DMSO-d_6) see Table 1.

Acetylation of **1**. Compound **1** was acetylated with Ac_2O (1 ml) and pyridine (1 ml) at room temp. overnight. After evapn of the reagents under vacuum and lyophilization, the undecaacetate was obtained. ^1H NMR ($CDCl_3$) for the glucose moiety, δ: 4.14 (1H, *dd*, $J = 11.5$; 6, H-6_aG), 4.08 (1H, *dd*, $J = 11.5$; 3.5, H-6_bG), 3.57 (1H, *m*, H-5G), 5.10 (1H, *t*, $J = 9.5$, H-4G), 3.98 (1H, *t*, $J = 9.5$, H-3G), 3.80 (1H, *dd*, $J = 7.5$; 9.5, H-2G), 4.43 (1H, *d*, $J = 7.5$, H-1G).

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DEFUSCIN, A NEW PHENOLIC ESTER FROM *DENDROBIUM FUSCESCENS*: CONFORMATION OF SHIKIMIC ACID*

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Key Word Index—*Dendrobium fuscescens*; Orchidaceae; defuscin; *n*-triacontyl *p*-coumarate; (−)-shikimic acid; conformation.

Abstract—Defuscin, a new phenolic ester shown to be *n*-triacontyl *p*-coumarate, and (−)-shikimic acid have been isolated from the whole plant of *Dendrobium fuscescens* Griff. The ^1H NMR spectrum of shikimic acid is indicative of the weighted average of its two half-chair conformers, the one with 4-OH and 5-OH in equatorial orientations being the major contributor.

INTRODUCTION

Dendrobium fuscescens Griff (Orchidaceae) [1, 2] is an epiphytic herb growing in Sikkim Himalayas, Khasia Mountains and Naga Hills at an altitude of 2300 m. It possesses pseudobulb stems with oblong lanceolate leaves and has purplish brown flowers. There is no previous report of work on this species though other *Denbrobium* species have been found to elaborate fluorenone derivatives [3–5], coumarins [6], a phenanthraquinone derivative [7], a polyoxygenated phenanthrene [8], alkaloids [9], spirophthalides [10], sesquiterpenes [11] and steroids [12]. From the whole plant, collected from the vicinity of Darjeeling, West Bengal, we have isolated and characterised a new phenolic ester designated defuscin (**1**), shown to be *n*-triacontyl *p*-coumarate, in addition to (−)-shikimic acid. To our knowledge, this is the first isolation of (−)-shikimic acid from an Orchidaceae plant.

RESULTS AND DISCUSSION

The petrol extract of the whole plant of *Dendrobium fuscescens* on extensive chromatography over silica gel furnished defuscin (**1**) from the petrol–ethyl acetate (17:3)

* Part V in the series 'On the Chemistry of Indian Orchidaceae Plants', For Part IV see ref [5].

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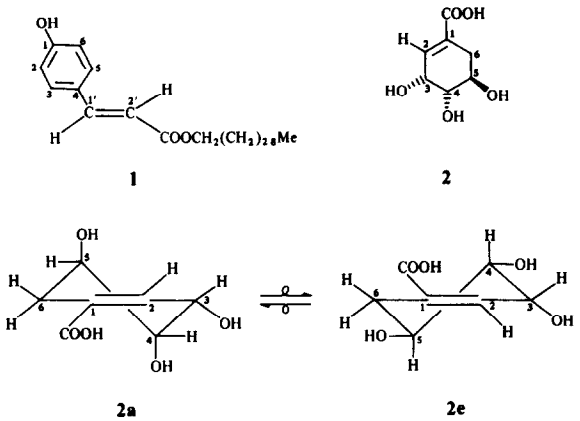

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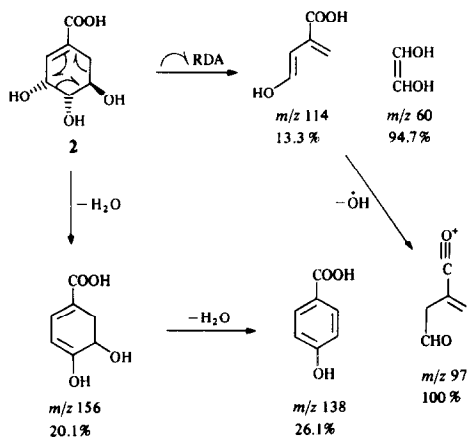

Scheme 1. Mass fragmentation of shikimic acid (**2**).

eluate fractions, crystallizing from chloroform–petrol as colourless needles (yield 0.007%), mp 101°, M^+ 584 ($C_{39}H_{68}O_3$); ν_{max}^{KBr} cm^{-1}: 3300–3400 (phenolic OH), 1680 (conjugated C=O). The alkali shift in the UV spectrum (see Expermental) and the appearance of a broad signal at δ5.80 (exchangeable with D_2O) in the ^1H NMR spectrum, are indicative of the presence of a phenolic OH group.

The ^1H NMR spectrum of (**1**) identifies the structure as *n*-triacontyl *p*-coumarate. A pair of doublets resonating at δ7.61 and 6.27 (1H each, J = 16.0 Hz) was assigned to H-1′ and H-2′ respectively, while another pair of doublets (2H each, J = 8.0 Hz) at δ7.39 (H-3 and H-5) and 6.83 (H-2 and H-6) appeared as an A_2B_2 system. The oxygen bearing methylene protons appeared at δ4.13 (2H, *t*, J = 7.0 Hz). A broad singlet at δ1.23, integrating for 56 protons, was assigned to all other methylene protons in the straight chain while the terminal methyl appeared as a triplet at δ0.91 (J = 6.0 Hz).

The mass spectrum of defuscin is fully consistent with its assigned structure. Characteristic peaks appeared at *m/z* (rel. intensity): 584 (M^+, 8.6), 438 (HO–CH_2–$(CH_2)_{28}$–Me, 4.4), 420 (CH_2=CH–$(CH_2)_{27}$–Me, 1.5), 164 (*p*–OH–C_6H_4–CH=CH–COOH, 100). The ester on alkaline hydrolysis followed by usual work-up yielded *p*-hydroxycinnamic acid and *n*-triacontanol. The structure (**1**) of defuscin was finally confirmed by direct comparison with an authentic sample prepared by esterification of *p*-hydroxycinnamic acid with *n*-triacontanol in CH_2Cl_2 in the presence of DCC.

The residue from the ethanol extract of the marc upon chromatography over silica gel afforded from EtOAc eluate fractions (−)-shikimic acid (**2**) [13] (0.009% yield) crystallizing from methanol–chloroform in the form of light greyish white solid, mp 184.5° (lit. [13] mp 184–185°); M^+ was missing in 70 eV MS due to facile dehydration); $[\alpha]_D$ −166.2°; ^1H NMR (CD_3OD, 200 MHz, δ) 6.83 (1H, *m*, $W_{1/2}$ = 9 Hz, H-2), 4.47 (1H, *t*, $J_{3,4} = J_{3,2}$ = 4 Hz, H-3), 4.05 (1H, 5 line *m*, $W_{1/2}$ = 20 Hz, H-5), 3.81 (1H, *dd*, $J_{4,5}$ = 8 Hz, $J_{4,3}$ = 4 Hz, H-4), 2.75 (1H, *dd*, $J_{6\alpha,5}$ = 5 Hz and $J_{6\alpha,6\beta}$ = 18.5 Hz, H-6α), 2.24 (1H, *dd*, $J_{6\beta,5}$ = 7 Hz and $J_{6\beta,6\alpha}$ = 18.5 Hz, H-6β). Since the spectrum was measured in CD_3OD no signals and no derived splitting were observed for the hydroxyls and carboxyl group. The analysis reported [13] in 1964 of the 60 MHz spectrum (in D_2O) of shikimic acid on the basis of 5 subdivided sections and computation did not give correct chemical shift value for H-4 due to overlapping with H-5, whereas in the present 200 MHz spectrum all the multiplets were well separated. The long range allylic coupling between H-2 and H-6 and the homoallylic coupling between H-6 and H-3, though not well resolved, produced broadening of each component signal of the protons. The coupling constants, $W_{1/2}$ values and multiplicities are due to the time-averaged conformation of (**2**) resulting from the conformational inversion between the two half-chair conformations (**2a**) and (**2e**)—the latter with 4-OH and 5-OH in equatorial orientation being the major contributor. This is in conformity with the axial-like orientation of H-6β as reflected in the value $J_{6\beta,5}$ = 7 Hz.

The free energy difference between (**2a**) and (**2e**) may be even less than that (0.9–2 kJ/mol) [14] between axial and equatorial conformers of cyclohexanol since (**2a**) possesses 4-OH(axial) H-6α(quasi-axial) and 5-OH(axial) H-3(quasi-axial) interactions whereas (**2e**) has only 3-OH(quasi-axial) H-5(axial) interaction. Earlier [13] conformation (**2a**) was not considered and shikimic acid was postulated to possess essentially the half-chair conformation (**2e**) with some deformation to a boat having the double bond at the middle of one side of the boat carrying 3-OH and 5-OH in equatorial-like and 4-OH in axial-like orientation. It is well-known that the boat conformation or even the more favourable half-boat conformation would be of much higher energy (> 6 kJ/mol) and hence may be present only in negligibly small concentration.

The assignment of the carbon signals in the ^{13}C NMR spectrum of (**2**) have been made on the basis of DEPT (distortionless enhancement by polarisation transfer) spectrum (CD_3OD, 50.30 MHz) clearly indicating the presence of one methylene, four methine and two quaternary carbon atoms. Apart from the signal for the carboxyl carbon at δ 170.18, C-2 being β to the carboxyl appeared relatively downfield at δ138.71 compared to C-1 (δ130.80). The methylene carbon (C-6) appeared most upfield at δ31.70. The hydroxyl bearing allylic carbon resonates at δ72.36 relatively downfield with respect to the other two hydroxyl bearing carbons C-4 and C-5. The signal at δ68.36 was assigned to C-4, being attached to two hydroxyl bearing carbons while the signal at δ67.31 was assigned to C-5 being attached to one hydroxyl bearing carbon. However, these two figures are interchangeable.

The 70 eV mass spectrum of shikimic acid, not reported earlier, lacked the molecular ion peak due to very facile elimination of the elements of water forming a species having m/z 156 (20.10). The genesis of the characteristic peaks has been shown in Scheme 1.

EXPERIMENTAL

General. Mps: uncorr. IR: KBr; ^{1}H NMR, TMS int. stand; MS: 70 eV; chromatography: silica gel (60–120 mesh); spots visualized in UV light and on exposure to I_2 vapour; Identity of each known compound was confirmed by direct comparison (mmp, IR, co-TLC) with an authentic sample.

Extraction. Air dried (800 g) and powdered whole plant of *Dendrobium fuscescens* was extracted in a Soxhlet apparatus with petrol and $CHCl_3$ successively for 48 hr each. The concentrates of the different extracts were chromatographed separately over silica gel (60–120 mesh), elution being carried out with solvent mixtures of increasing polarity.

Isolation of defuscin (**1**) *from petrol extract.* The concentrate of the petrol extract (4 g) was chromatographed over silica gel (60–120 mesh). The white solid obtained from petrol–EtOAc (17:3) eluate fractions after rechromatography over silica gel (100–200 mesh) followed by crystallization from $CHCl_3$–petrol, afforded white needle-shaped crystals of defuscin (**1**) (40 mg), mp. 101°, R_f 0.25, MeOH–$CHCl_3$ (1:99). ν_{max}(KBr): 3300–3400 cm^{-1}, 1710, 1680, 1605, 1514, 1470, 1170, 980, 830, 719; λ_{max} (EtOH): 212 nm (log ε 3.96), 228 (4.00), 312 (4.32), $\lambda_{max}^{EtOH+NaOH}$: 207 (3.75), 242 (3.8), 312 (3.92), 359 (4.43).

The $CHCl_3$ extract did not yield any crystalline compound. The marc left after petrol and $CHCl_3$ extraction of the dried and powdered whole plant of *D. fuscescens* was immersed in EtOH (90%) for 20 days. The gummy brown residue (3 g) obtained from EtOH extract was chromatographed over silica gel (60–120 mesh), elution being carried out with solvent and solvent mixtures of increasing polarity.

Isolation of (−)-shikimic acid (**2**) *from* EtOH *extract.* The solid obtained from EtOAc eluate fractions of the chromatogram was subjected to rechromatography over silica gel (100–200 mesh) followed by crystallization from MeOH–$CHCl_3$ to afford greyish white crystals of (−)shikimic acid (**2**) (60 mg), mp 184.5° R_f 0.7, $CHCl_3$–MeOH (3:7), $[\alpha]_D$ −166.2°; ν_{max}^{KBr}: 3480 cm^{-1}, 3380, 3220, 1680, 1645, 1452, 1290, 1070, 930.

Hydrolysis of defuscin (**1**): *isolation of* n-*triacontanol and* p-*hydroxycinnamic acid.* To defuscin (**1**) (20 mg) taken in $CHCl_3$ (2 ml) was added a soln of 5% KOH in MeOH (1.5 ml) and the mixture was refluxed for 2 hr, concd. under red. pres., diluted with H_2O (5 ml) and extracted with Et_2O. The ethereal extract on concn yielded *n*-triacontanol, crystallizing from $CHCl_3$–MeOH mixture (yield 5 mg), mp 85°. The aq. layer was acidified with dil. HCl and extracted with $CHCl_3$. The organic layer upon usual work-up yielded *p*-hydroxycinnamic acid in colourless crystals (yield 10 mg), mp 210°; (MeOH–$CHCl_3$).

Synthesis of defuscin (**1**). A soln of *N*,*N*′-dicyclohexyl carbodiimide (DCC) (20 mg) in dry CH_2Cl_2 (3 ml) was added dropwise during a period of 30 min at 0° to a well-stirred soln of of *p*-coumaric acid [15] (10 mg) and *p*-triacontanol (23 mg) in dry CH_2Cl_2 (4 ml). The mixture was further stirred for 90 min at 25°. The residue obtained upon evapn of the solvent was treated with EtOAc (6 ml), and the separated dicyclohexyl urea was filtered. The filtrate on concn yielded defuscin (= *n*-triacontyl *p*-coumarate), mp 101°, yield 70%, identical (mmp, IR, co-TLC) with the natural sample.

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