EXPERIMENTAL

The ^{13}C NMR (20 MHz) spectra were run in $CDCl_3$/TMS. The complete proton noise decoupled spectra as well as APT spectra were run. Column chromatographic separations were done on glass columns using silica gel (Merck) as the stationary phase. TLC were performed with silica gel G (HF_{254}) plates using C_6H_6–EtOAc (43:7).

Extraction and separation. The above ground parts of *K. tribuloides* (800 g) was defatted (C_6H_{14}) and subsequently extracted with 90% EtOH for 30 hr in a Soxhlet. The concd extract was hydrolysed in the usual way with 10% H_2SO_4 for 2 hr. The crude sapogenin obtained in the usual way was dried and extracted with hexane. The hexane-soluble dark green sapogenin mixture was chromatographed on an alumina column and the benzene eluate gave a mixture from which tigogenin [10], mp 198–200°, acetate, mp 203–205° and stigmasterol, mp 168–169°; acetate, mp 142–144°, were subsequently isolated by PLC. The $CHCl_3$–MeOH eluate gave substance A which was crystallized several times from MeOH (0.8 g), mp 250–258°; diacetate, mp 228–232°.

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CREPIDATIN, A BIBENZYL DERIVATIVE FROM THE ORCHID *DENDROBIUM CREPIDATUM*

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Key Word Index—*Dendrobium crepidatum*; Orchidaceae; crepidatin; bibenzyl derivative.

Abstract—Crepidatin, a new bibenzyl derivative, was isolated from the orchid *Dendrobium crepidatum*. Its structure was determined from spectral data.

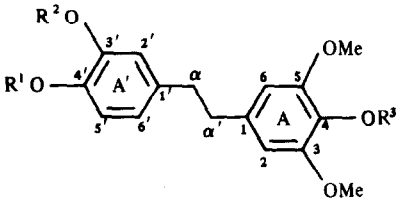
INTRODUCTION

From a series of Indian orchids we reported [1–3, 5–25] earlier the isolation of a number of compounds which represent several structural types like bibenzyls [1, 2], phenanthrenes [3–9] (both monomeric and dimeric), phenanthropyrans [10] and pyrones [11], 9,10-dihydrophenanthrenes [12–14] (both monomeric and dimeric), 9,10-dihydrophenanthropyrans [15–20] and pyrones [11, 15–17, 21], triterpenoids [22, 23] and steroids [24, 25]. The orchid *Dendrobium crepidatum* was chemically investigated by Leander *et al.* [26, 27] who reported the isolation of several alkaloids from this plant. Further chemical investigation of this orchid has now afforded a new bibenzyl derivative, designated as crepidatin, which was shown to have the structure **1a** from the following evidence.

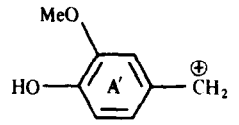
RESULTS AND DISCUSSION

Crepidatin, $C_{18}H_{22}O_5$ (M^+ 318), mp 99°, showed typical benzenoid UV absorptions, λ_{max} 211 and 279 nm ($\log\varepsilon$ 4.51 and 3.61). The phenolic nature of the compound was indicated by its characteristic colour reactions, alkali-induced bathochromic shifts of its UV maxima [λ_{max} 218 and 296 nm ($\log\varepsilon$ 4.34 and 3.59)] and by its IR band at 3342 cm^{-1}. Crepidatin formed a monoacetyl derivative, $C_{20}H_{24}O_6$ (M^+ 360), mp 64°, with acetic anhydride and pyridine, confirming the presence of a single phenolic hydroxyl group.

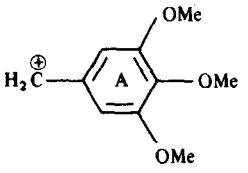
The ^1H NMR spectrum of crepidatin showed signals for a single phenolic hydroxyl proton at δ5.47 (disappeared on deuterium exchange), four aromatic methoxyl groups at δ3.81 (9H, *s*) and 3.83 (3H, *s*), four equivalent benzylic methylene protons at δ2.82 (4H, *s*), typical of the



1a $R^1 = H, R^2 = R^3 = Me$
1b $R^1 = Ac, R^2 = R^3 = Me$
1c $R^1 = R^3 = Me, R^2 = H$
1d $R^1 = R^3 = Me, R^2 = Ac$
1e $R^1 = R^3 = H, R^2 = Me$
1f $R^1 = R^3 = Ac, R^2 = Me$



m/z 137 (**a**)



m/z 181 (**b**)

four benzylic methylene protons of a bibenzyl derivative [1, 2], and five aromatic protons. Two of these aromatic protons appeared as a sharp singlet at δ6.35 for the two equivalent protons H-2 and H-6 of a 3,4,5-trimethoxy benzyl residue, while the chemical shifts and the splitting patterns of the remaining three aromatic protons resonating at δ6.60 (1H, *d*, $J = 1.9$ Hz), 6.69 (1H, *dd*, $J_1 = 8$ Hz and $J_2 = 1.9$ Hz) and 6.84 (1H, *d*, $J = 8$ Hz) are consistent with their association with a 3,4-oxygenated benzyl moiety. These three aromatic protons may thus be attributed to H-2′, H-6′ and H-5′ respectively of crepidatin.

The mass spectrum of crepidatin showed intense peaks at *m/z* 137 (ion-fragment **a**) and 181 (ion-fragment **b**, base peak), which not only supported the bibenzyl formulation of the compound but also indicated the distribution of the hydroxyl and methoxyl groups in the two benzene rings.

The above spectral data suggests that crepidatin is a structural isomer of erianin (**1c**) [1] (isolated earlier in our laboratory from the orchid *Eria carinata*) having hydroxyl and methoxyl groups interchanged between C-3′ and C-4′. This was finally confirmed by the ^{13}C NMR spectral data of crepidatin and its acetate (Table 1). The degree of protonation of each carbon atom was determined by DEPT experiments and the assignments of the carbon chemical shifts were made by comparison with the δ_c values of erianin acetate (**1d**) [1], moscatilin (**1e**) [2] and structurally similar compounds [28] taking into consideration the known additive parameters of the functional groups in the benzenoid system. Thus the δ_c values of C-1, C-2, C-3, C-4, C-5, C-6, C-α and C-α' of crepidatin and crepidatin acetate appeared essentially at the same positions as the corresponding carbon atoms of erianin acetate (**1d**) [1] and other structurally similar compounds [28] containing 3,4,5-trimethoxy phenyl ethyl moiety, while those of C-1′, C-2′, C-3′, C-4′, C-5′ and C-6′ constituting ring A′ of crepidatin are almost identical with the δ_c values of the corresponding carbon atoms of moscatilin (**1e**). The above observations thus confirm the structural identity of ring A of crepidatin with ring A of erianin (**1c**) and that of ring A′ with the ring A′ of moscatilin (**1e**). This was further confirmed by a comparison of the δ_c values of crepidatin acetate and erianin acetate (**1d**). They differed essentially in regard to their ring-A′ carbon resonances. The upfield shift of C-6′ and the downfield shift of C-1′ of crepidatin acetate by ~6 ppm compared to the corresponding carbon atoms of erianin acetate can only be rationalized by placement of a methoxyl group at C-3′ and a hydroxyl group at C-4′ in crepidatin (**1a**) as against erianin (**1c**) having these groups interchanged between these positions.

Table 1. ^{13}C NMR spectral data of crepidatin (**1a**), crepidatin acetate (**1b**), erianin acetate (**1d**) and moscatilin (**1e**)

	Chemical shifts (δ values)*			
C	**1a**	**1b**	**1d**†	**1e**
1	137.38	137.66	137.48	132.84[a]
2	105.22	105.31	105.40	105.19
3	152.81	152.69	153.0	146.77[b]
4	135.84	136.02	136.20	133.53[a]
5	152.81	152.69	153.0	146.77[b]
6	105.22	105.31	105.40	105.19
α	38.46[a]	37.81[a]	38.82[a]	38.28[c]
α′	37.48[a]	37.45[a]	36.96[a]	37.75[c]
1′	133.35	140.11	134.27	132.76[a]
2′	111.02	112.60	122.40	111.18
3′	146.09	150.39	139.50	146.16[b]
4′	143.61	136.76	149.30	143.69
5′	114.04	122.06	112.28	114.07
6′	120.83	120.22	126.68	120.98
OMe (C-3)	55.82	55.67	56.05	56.15
OMe (C-4)	60.64	60.36	60.86	—
OMe (C-5)	55.82	55.67	56.05	56.15
OMe (C-3′ or C-4′)	55.87 (C-3′)	55.39 (C-3′)	55.99 (C-4′)	55.76
OCOMe	—	168.64 20.15	169.12 20.68	—

*The δ values are in ppm downfield from TMS: $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.

†In the light of our recent studies we have revised our earlier assignments [1] of some of the nonprotonated carbon atoms of **1d** as shown here.

[a–c]Values in the same column are interchangeable.

Crepidatin (**1a**) is thus a new addition to the growing list of naturally occurring bibenzyl derivatives. In the light of the recent observation of the antimitotic properties of structurally similar bibenzyl derivatives [28], crepidatin may be studied for similar biological properties.

EXPERIMENTAL

Mps: uncorr. Silica gel (60–100 mesh) was used for CC and silica gel G for TLC. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra in KBr discs. ^1H and ^{13}C NMR were measured at 250 and 300 MHz and 75 MHz respectively in $CDCl_3$ using TMS as int. standard, chemical shifts are expressed in δ values. MS were recorded with a direct inlet system at 70 eV. All the analytical samples were routinely dried over P_2O_5 for 24 hr *in vacuo* and were tested for purity by TLC and mass spectrometry. Na_2SO_4 was used for drying organic solvents and the petrol used was bp 60–80°.

Isolation of crepidatin (**1a**). Air-dried powdered whole plant of *D. crepidatum* (2 kg) was soaked in MeOH for 3 weeks. The methanolic extract was drained and concd under red. pres. to *ca* 75 ml, diluted with H_2O (500 ml) and extracted with Et_2O. The Et_2O extract was fractionated into acidic and non-acidic fractions with 2 M aq. NaOH soln. The aq. alkaline soln. was acidified in the cold with conc. HCl and the liberated solids extracted with Et_2O, washed with H_2O, dried, and the solvent removed. The residue was chromatographed. The petrol–EtOAc (20:1) eluate, on evapn, gave a semi-solid mass containing mostly **1a**. Repeated chromatography of this material finally afforded pure **1a**, (0.3 g), crystallized in needles from petrol–EtOAc mixture, mp 99°. It gave blue coloration with phosphomolybdic acid reagent on exposure to NH_3. (Found: C, 67.85; H, 6.89. $C_{18}H_{22}O_5$ requires: C, 67.92; H, 6.91%). IR ν_{max} cm^{-1}: 3342 (OH), 1590, 1510, 898, 888, 851, 811, 799 and 781 (phenyl nucleus); MS *m/z* (rel. int.): 318 (M^+, 52), 182 (29), 181 (100), 167 (7), 148 (9), 138 (9), 137 (64), 138 (9), 122 (9), 94 (11) and 77 (9).

Crepidatin (**1a**) was aceylated with Ac_2O–pyridine in the usual manner to give **1b**, crystallized from petrol–EtOAc, mp 64° (found: C, 66.71; H, 6.60. $C_{20}H_{24}O_6$ requires: C, 66.67; H, 6.67%). UV λ_{max} nm: 212 and 272 nm (log ε 4.39 and 3.41); IR ν_{max} cm^{-1}: 1240, 1255 and 1759 (OAc), 1602, 1591, 1505, 845, 825, 815 and 776 (phenyl nucleus); ^1H NMR: δ2.22 (3H, *s*; OAc), 2.85 (4H, *s*; H_2-α and H_2-α'), 3.75 (3H, *s*; 1 × OMe), 3.80 (6H, *s*; 2 × OMe), 3.81 (3H, *s*; 1 × OMe), 6.32 (2H, *s*; H-2 and H-6), 6.68 (1H, *br s*; H-2'), 6.74 (1H, *br d*; J = 9 Hz; H-6') and 6.92 (1H, *d*, J = 9 Hz; H-5').

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