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Arundinol, A New Triterpene from the Orchid *Arundina bambusifolia*

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Arundinol, a new triterpene, isolated from the orchid *Arundina bambusifolia* was shown to be cycloartenyl 3β-*p*-hydroxy-*trans*-cinnamate (1a) from spectral and chemical evidence

WE reported earlier the isolation of a few triterpenoids[1,2] and structurally related steroids[3-5], besides a fairly large number of stilbenoids from a series of Indian orchids. Our continued search for new phytochemicals from the same source has resulted in the isolation of yet another new triterpenoid, designated as arundinol, from the orchid *Arundina bambusifolia*. It was shown to have the structure **1a** from the following spectral and chemical evidence.

Results and Discussion

Elemental analysis of arundinol (**1a**), m.p. 245°, $[\alpha]_D$ +3.52° (MeOH), corresponded to a molecular formula $C_{39}H_{56}O_3$ which was confirmed by its chemical-ionisation mass spectrum showing peak at *m/z* 573 $[M+1]^+$. It, however, did not show the molecular ion peak in its electron-impact mass spectrum which, instead, exhibited a peak at *m/z* 408 $[M-C_9H_8O_3]$ at the highest mass region corresponding to the loss of *p*-hydroxycinnamic acid.

The presence of a *p*-hydroxy-*trans*-cinnamic ester moiety in **1a** was indicated by its spectral data[1,2]: λ_{max}(EtOH) 213, 227, 255 and 313 nm (log ε 3.56, 3.55, 3.29 and 3.63); large alkali-induced bathochromic shifts, λ_{max} (EtOH−0.1*M* NaOH) 230, 310 and 365 nm (log ε 3.43, 3.36 and 3.74)]; ν_{max} 3 220 (OH), 1 680 (conjugated C=O), 980 (*trans* −CH=CH−) and 830 cm^{-1} (1,4-disubstituted benzene); ^{1}H nmr δ (d_6-acetone) 9.06 (1H, s, disappeared on deuterium exchange; phenolic OH), 7.58 and 6.91 (each 2H, d, *J* 9 Hz; four aromatic protons of the *p*-disubstituted-benzene moiety) and 7.65 and 6.39 (each 1H, d, *J* 16 Hz; *trans*-olefinic protons of the α,β-unsaturated ester function). The ^{1}H nmr spectrum of **1a** also showed signals at δ 5.15 (1H, m, trisubstituted-olefinic proton), 4.70 (1H, m, *Wh*/2 16 Hz; ester methine proton), 1.68 and 1.62 (each 3H, s, two vinylic methyls associated with the moiety −CH=CMe_2), 0.66 and 0.46 (each 1H, d, *J* 4 Hz, methylene protons of a tetrasubstituted-cyclopropane ring), 0.90 and 0.96 (each 3H, s, 2× −C−Me), 0.99 (3H, d, *J* 6.5 Hz, CH−Me), 1.02 and 1.04 (each 3H, s, 2× −C−Me). The above spectral data coupled with its characteristic mass spectral fragmentation giving rise to peaks at *m/z* 69 ($\overset{+}{C}H_2$−CH=CMe_2), 297 (ion-fragment formed by the loss of *p*-hydroxycinnamic acid and cleavage of 17−20 bond), besides those at *m/z* 164 (*p*-hydroxycinnamic acid) and 147 (*p*-hydroxycinnamoyl ion) are in conformity with the structure **1a** for arundinol. The equatorial orientation of the ester function followed from the large *Wh*/2 value of the signal of the ester methine proton at C-3

Treatment of **1a** with acetic anhydride and pyridine gave the acetyl derivative **1b**, $C_{41}H_{58}O_4$, m.p. 145°, which on catalytic hydrogenation over 10% Pd−C afforded the corresponding tetrahydro derivative **1d**, $C_{41}H_{62}O_4$, the ir and ^{1}H nmr spectral data of which corresponded to the reduction of the two olefinic double bonds of **1b**. The ^{1}H nmr signal of H-3 of **1d** (δ 4.57, *Wh*/2 15 Hz) is shifted upfield by 0.13 ppm due to the reduction of the olefinic double bond of the *p*-hydroxycinnamic ester moiety[1]. The *Wh*/2 value of the signal further confirmed the assigned stereochemistry at C-3 of **1a**. None of **1b** and **1d** showed molecular ion in their respective EI-mass spectra which exhibited peaks at *m/z* 408 $(M-C_{11}H_{10}O_4)$ and 410 $(M-C_{11}H_{12}O_4)$ respectively at the highest mass region.

More compelling evidence in support of the structure **1a** was provided by the ^{13}C nmr spectral data of its more soluble derivatives **1b** and **1d** (Table 1). The degree of protonation of the carbon atoms was determined by DEPT experiments, and the assignments of the carbon chemical shifts were made by comparison with the δ_c values of structurally related compounds[1,2,5-10] taking into consideration the additive parameters of the functional groups and the effect caused by minor structural changes. Thus, the δ_c values of the carbon atoms of the aromatic ester moiety of **1b** are essentially the same as those of *p*-acetoxy-*trans*-cinnamoyl residue of the acetyl derivative of rubicoumaric acid[10] and are also comparable with those of the *p*-hydroxy-*trans*-cinnamoyl moiety of 24-methylene cycloartanyl *p*-hydroxyl-*trans*-cinnamate[1] taking into considera-

TABLE 1—CARBON CHEMICAL SHIFTS OF COMPOUNDS **1b** AND **1d**

C	Chemical shifts (δ)*		C	Chemical shifts (δ)*	
	1b	**1d**		**1b**	**1d**
1	31.51	31.50	20	35 76	36 03
2	26 78	26.63	21	18 12[a]	18.23[a]
3	80.73	80 80	22	36.23	36.39
4	39.57	39.45	23	24.83	24.01
5	47.09	47.10	24	125 15	39.45
6	20.81	20.80	25	130.71	27.89
7	28.00	28 03	26	17.49[a]	22.42
8	47 69	47.69	27	25 56	22 68
9	20.06	20.09	28	19.16[a]	19 19[a]
10	25 89	25.91	29	25 36	25.28
11	25.69	25.70	30	15.18	15 07
12	35.42	35.45	C-1′	168.92	172 40
13	45.18	45.20	C-2′	119 0	36.03
14	48 69	48 72	C-3′	142.96	30 37
15	32.75	32.80	C-1″	132 20	138 07
16	26.41	26 45	C-2″ C-6″	129.0	129.1
17	52.15	52 30	C-3″ C-5″	121 92	121 35
18	17.90[a]	17.84[a]	C-4″	150.88	149.03
19	29.63	29.62	OCOMe	166.52	169.32
				20.95	23.94

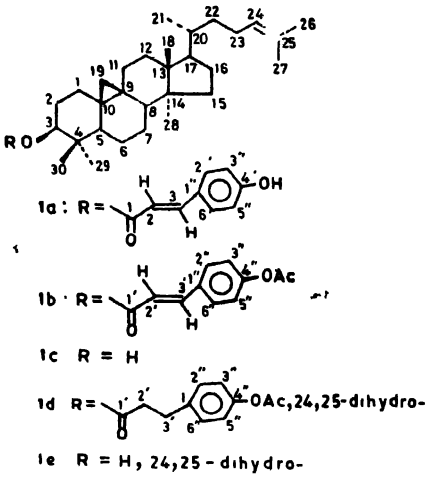
*Values are in ppm downfield from TMS ; $\delta_{(TMS)}=\delta_{(CDCl_3)}+76.9$ ppm.

[a]Values in the same column are interchangeable.

tion the different additive parameters of the hydroxy and acetoxy groups. The δ_c values of the corresponding carbon atoms of **1d** are also in conformity with reduction of olefinic double bond of the ester moiety. Confirmation of the structure and stereochemistry of the triterpenoid part of **1a** was provided by the fact that while the δ_c values of C-1 – C-30 of **1d** are almost identical with those of the corresponding carbon atoms of cycloartanyl acetate[9], the chemical shifts of these carbon atoms of **1b** differ essentially from those of the above compounds only in respect of C-24, C-25, C-26 and C-27 due to the presence of a double bond between C-24 and C-25 in **1b**[9].

Structure **1a** for arundinol was finally confirmed by the following chemical evidence. Alkaline hydrolysis of **1a** with 10% aqueous methanolic NaOH under reflux for 4 h afforded an acid, $C_9H_8O_3$, m.p. 212° d, and a neutral compound, $C_{30}H_{50}O$ (M^+ at *m/z* 426), m.p. 85°, $[\alpha]_D+46°$ ($CHCl_3$). The hydrolysis of **1a** was, however, incomplete due to the reduced carbonyl character of the *p*-hydroxycinnamic ester moiety. Under identical conditions, **1d**, on the otherhand, was completely hydrolysed also to an acid, $C_9H_{10}O_3$, m.p. 124°, and another neutral compound, $C_{30}H_{52}O$ (M^+ at *m/z* 428), m.p. 100°, $[\alpha]_D+44°$ ($CHCl_3$). The acids obtained from **1a** and **1d** were shown to be identical in all respects with authentic samples of *p*-hydroxy-*trans*-cinnamic and *p*-hydroxyphenylpropionic acids respectively. The physical constants and the spectral data of the neutral compound derived from **1b** and those of the compound obtained from **1d** compare excellently with those of cycloartenol (**1c**)[11] and cycloartanol (**1e**)[11-14] respectively, indicating identity in each case, although direct comparison could not be made due to nonavailability of authentic samples.

The structure of arundinol is thus established as cycloartenyl 3β-*p*-hydroxy-*trans*-cinnamate (**1a**). Biogenetically, it is elaborated utilising the precursors of the stilbenoids and triterpenoids/steroids, the former constituting the major chemical constituents of the orchidaceae plants.



Experimental

M.ps. are uncorrected. Silica gel (100 – 200 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 nmr spectra were run at 300 MHz in $CDCl_3$ (except for **1a** at 100 MHz in d_6-acetone) using TMS as the internal standard and ^{13}C nmr at 75 MHz in $CDCl_3$ with the same internal standard, carbon chemical shifts being downfield from TMS : $\delta_{(TMS)}=\delta_{(CDCl_3)}+76.9$ ppm. Mass spectra were recorded in an instrument equipped with a direct inlet system and operating at 70 eV ; NH_3 was used as the carrier gas in chemical ionisation MS. Figures in the first bracket attached to the *m/z* values represent rel. int. of peaks. All analytical samples were routinely dried over P_2O_5 for 24 h under reduced pressure and were tested for purity by tlc and MS. Dry Na_2SO_4 was used for drying organic solvents, and petrol used had b.p. 60 – 80°.

Isolation of **1a**: Air-dried powdered whole plant materials of *A. bambusifolia* (2 kg) was kept soaked in MeOH (6 dm^3) for 3 weeks. The MeOH extract was concentrated (100 ml) under reduced pressure, diluted with H_2O (500 ml) and the liberated solid was extracted with Et_2O. The Et_2O extract was fractionated into acidic and neutral parts with 2*M* aqueous NaOH solution. The aqueous alkaline solution was acidified with concentrated HCl in the cold. The liberated solid was extracted with Et_2O, washed with H_2O, dried and the solvent was removed. The residue was chromatographed. The petrol-EtOAc (20 : 1) eluate gave **1a** (0.04 g) which was crystallised

from the same solvent mixture, m.p. 245° (Found : C, 81.80 ; H, 9.76. $C_{39}H_{56}O_3$ requires : C, 81.82 ; H, 9.79%) ; $[\alpha]_D$ +3.52° (MeOH) ; ν_{max} 3 220 (OH), 3 012 (cyclopropane ring), 1 680 (conjugated >C=O), 980 (*trans* −CH=CH−), 830 (1,4-disubstituted benzene) and 1 665 cm^{-1} (trisubstituted olefin) ; MS (EI) similar to those of **1b** ; MS (CI) *m/z* 573 (M^++1).

Compound **1a** was acetylated with Ac_2O and pyridine in the usual manner to give **1b**, crystallised from petrol-EtOAc (5 : 1) mixture, m.p. 145° (Found: C, 80.10 ; H, 9.42. $C_{41}H_{58}O_4$ requires : C, 80.13 ; H, 9.44%) ; λ_{max} 219, 283 and 288 nm (log ε 4.14, 4.39 and 4.37) ; ν_{max} 3 040 (cyclopropane ring), 1 763 and 1 272 (OAc), 1 698 (conjugated ester C=O), 1 622 (olefinic double bond), 985 (*trans* −CH=CH−) and 840 cm^{-1} (1,4-disubstituted benzene) ; ^1H nmr δ 7.54 and 7.12 (each 2H, d, *J* 8.4 Hz, H-3″, H-5″ and H-2″, H-6″), 7.64 and 6.40 (each 1H, d, *J* 15.9 Hz, H-3′ and H-2′), 5.10 (1H, m, H-24), 4.71 (1H, m, *Wh*/2 15.6 Hz, H-3), 2.30 (3H, s, OAc), 1.65 and 1.58 (each 3H, s, vinylic CH_3), 0.89 (3H, d, *J* 6.5 Hz, CHMe), 0.97 (6H, s, 2× −C−Me), 0.90 and 0.88 (each 3H, s, 2× −C−Me) and 0.60 and 0.36 (each 1H, d, *J* 4.2 Hz, H_2-19) ; MS (EI) *m/z* (rel. int.) 408 [M^+−(CH_2=C=O)−($C_9H_8O_3$)] (6.3), 393 (2.3), 365 (1.4), 339 (2.4), 297 (1), 203 (5.1), 189 (10), 175 (6), 164 (12), 147 (62), 123 (17), 107 (29.5), 95 (34), 91 (35.6), 69 (100), 55 (47.2) and 43 (72.7).

Catalytic hydrogenation of **1b** : A solution of **1b** (0.04 g) in MeOH−EtOAc (1 : 1 ; 25 ml) was added to 10% Pd−C (0.02 g) and the mixture was stirred in an atmosphere of H_2 for 4 h. The catalyst was then filtered off and the filtrate on evaporation gave **1d** (0.038 g) as a glassy solid (Found : C, 79.59 ; H, 10.01. $C_{41}H_{62}O_4$ requires : C, 79.61 ; H, 10.03%) ; ν_{max} 3 030 (cyclopropane ring), 1 763 and 1 260 (OAc), 1 723 (CO_2R), 1 610 and 795 cm^{-1} (phenyl nucleus) ; ^1H nmr δ 7.21 and 6.70 (each 2H, d, *J* 8.4 Hz, H-2″, H-6″ and H-3″, H-5″), 4.57 (1H, m, *Wh*/2 15 Hz, H-3), 2.95 and 2.63 (each 2H, t, *J* 7.5 Hz, H_2-2′ and H_2-3′), 2.28 (3H, s, OAc), 0.95 (3H, s, −C−Me), 0.89, 0.87 and 0.86 (5 C-methyls), 0.78 (3H, s. −C−Me), 0.56 and 0.33 (each 1H, d, *J* 4.2 Hz, H_2-19) ; MS (EI) *m/z* (rel. int.) 410 [M^+−(CH_2=C=O)−($C_9H_{10}O_3$)] (11.5), 395 (6.1), 215 (1.5), 203 (10.9), 187 (5.6), 175 (16.9), 166 (3), 161 (8.9), 149 (16.6), 135 (16.3), 123 (28.5), 121 (26.7), 107 (100), 95 (76), 81 (35.1), 69 (56), 57 (48) and 55 (50.4).

Alkaline hydrolysis of **1a** : A solution of **1a** (0.035 g) in 10% aqueous methanolic NaOH (15 ml) was refluxed for 4 h. MeOH was then removed under reduced pressure. The residue was diluted with water, extracted with Et_2O, dried and the solvent was removed. The residue was chromatographed. The petrol-EtOAc eluate (50 : 1) gave **1c** (0.02 g), crystallised from petrol-EtOAc (4 : 1), m.p. 85°, $[\alpha]_D$ +46° ($CHCl_3$) ; ν_{max} 3 020 (cyclopropane), 3 400 (OH) and 1 655 cm^{-1} (trisubstituted double bond) ; ^1H nmr δ 5.09 (1H, app. t, *J* 6.4 Hz, H-24), 3.28 (1H, m, *Wh*/2 18 Hz, H-3), 1.68 and 1.61 (each 3H, s, two vinylic CH_3), 0.81−0.96 (5 C-methyls) and 0.55 and 0.33 (each 1H, d, *J* 4.2 Hz, H_2-19). Elution of the column with petrol-EtOAc (20 : 1) gave unchanged **1a** (0.005 g). The aqueous alkaline solution left after removal of **1c** was acidified with concentrated HCl in the cold and the liberated solid was extracted with Et_2O. The residue after removal of Et_2O was chromatographed. The petrol-EtOAc (3 : 1) eluate gave *p*-hydroxy-*trans*-cinnamic acid (0.006 g) which was crystallised from petrol-EtOAc, m.p. 212° d.

Alkaline hydrolysis of **1d** : A solution of **1d** (0.04 g) in 10% aqueous methanolic NaOH (20 ml) was refluxed for 4 h. MeOH was then removed under reduced pressure. The residue was diluted with water, extracted with Et_2O, dried and the solvent was evaporated. The product was chromatographed and the petrol-EtOAc (50 : 1) eluate gave **1e** (0.022 g) which was crystallised from petrol-EtOAc (4 : 1), m.p. 100°, $[\alpha]_D$ +44° ($CHCl_3$) ; ν_{max} 3 350 cm^{-1}(OH) ; ^1H nmr δ 3.27 (1H, m, H-3), 0.80−0.96 (7 C-methyls) and 0.55 and 0.33 (each 1H, d, *J* 4.2 Hz, H_2-19). The aqueous alkaline solution after removal of **1e** was acidified with concentrated HCl in the cold and the liberated solid was extracted with Et_2O. Evaporation of the solvent gave a solid which was chromatographed. The petrol-EtOAc (3 : 1) eluate gave *p*-hydroxyphenylpropionic acid (0.008 g) which was crystallised from petrol-EtOAc, m.p. 124°.

Chromatography of the neutral fraction of the original methanolic extract of *A. bambusifolia* also gave a further quantity (0.01 g) of **1a**.

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