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Note

New taraxerane esters from *Hibiscus schizopetalus* leaves

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Two new triterpene esters, named 22-hydroxytaraxeryl acetate **1** and 22-hydroxytaraxeryl-*cis*–p-coumarate **2**, have been isolated from the leaves of *Hibiscus schizopetalus*. Their structures have been determined by spectroscopic methods and chemical reactions.

Keywords: *Hibiscus schizopetalus*, taraxerane, hydroxyt-araxeryl-acetate, hydroxytaraxeryl-*cis*-cinnamate, anticancer property

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Hibiscus schizopetalus (Wall.) Hook f.[1,2] belongs to the family Malvaceae. These plants have been used as ingredients in many of the Ayurvedic preparations. The *Hibiscus schizopetalus* is one of the least phytochemically examined species of the genus, Hibiscus. In this communication, we report the isolation and characterization of two new triterpene esters from the petroleum ether extract of leaves of *H. schizopetalus*. Such compounds are known for their potential anticancer activities.

Results and Discussion

Shade dried and powdered leaves of *H. schizopetalus* was extracted with pet. ether in a Soxhlet extractor for 36 hr. The extract (5L) was concentrated under reduced pressure and column chromatographed over silica gel. It was then eluted with pet. ether followed by pet. ether-ethyl acetate mixtures of varying composition. Two compounds were isolated.

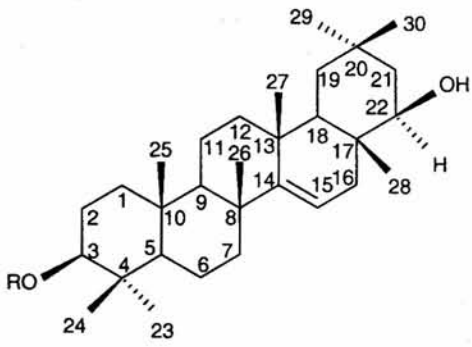
The compound **1** was obtained as a colourless powder on elution with pet. ether-ethyl acetate mixture in the ratio 9:1. It gave a single spot on TLC analysis. It gave positive colour reactions with Liebermann-Burchard reagent, vanillin-sulphuric acid reagent and anisaldehyde-sulphuric acid reagent for triterpenoid[3]. It decolourised Baeyer's reagent indicating unsaturation in the molecule.

The IR spectrum of **1** exhibited a strong absorption at 1724 cm^{-1} indicating ester carbonyl group. A broad absorption between 3716-3265 cm^{-1} showed the presence of an –OH group. Other bands appeared are 1251 cm^{-1} (C–O–C str), 1377 and 1467 cm^{-1} (C–H bending), 1649 cm^{-1} (C=C str) and 2922 and 2852.5 cm^{-1} (C–H str, asym and sym).

The ^{1}H- and ^{13}C-NMR spectra of the compound exhibited a pentacyclic triterpenoid pattern. The spectral data is similar to that of taraxeryl acetate[4,5] in major aspects and there are additional features indicating the presence of hydroxyl functions. The DEPT-90° and DEPT-135° spectra accounts for the presence of six methine (CH) carbons, nine methylene (CH_2) carbons and eight methyl (CH_3) carbons. The additional –OH group present in the taraxeryl ring skeleton of the compound **1** than that reported for taraxeryl acetate can be ascertained by IR absorption band at 3907-3265 cm^{-1} and from ^{1}H- and ^{13}C- NMR signals. A broad signal at δ 5.12 indicated the presence of –OH groups. The ^{1}H- and ^{13}C-NMR data showed that there were two CH groups with chemical shifts characteristic of an attached oxygen functionality. The signal at δ 4.45 (dd, J = 6.6, 9.6 Hz) can be attributed to the α-oriented carbinol proton at C-3. The double doublet can be justified as due to the magnetically non-equivalent hydrogens at C-2. The signal at δ 3.64 (t, J = 6.6 Hz) was attributed to the H at C-22 (ref. 6). The ^{1}H NMR signal at 5.53 (dd, J = 8.1, 3 Hz) was assigned to olefinic H at C–15 interacting with H on C-16 and C-18.

The ^{13}C signal at δ 158 ppm and 117 ppm can be attributed to C-14 and C-15, respectively. The ^{13}C NMR signal at 170.9 ppm was assigned to carbonyl carbon of the acyl group and 21.3 ppm was attributed to acetyl methyl carbon (**Table I**).

In the mass spectrum, the molecular ion signal at m/z = 484 is not obtained. Usually the ion with highest m/z value will be the (M^+ - 18) peak[7]. In the present case the peak at m/z = 466 was attributed to (M^+-18) peak. The other peaks at m/z values 451, 407, 344 and the base peak at 203 were quite consistent with the proposed structure for compound **1**. The ion fragment



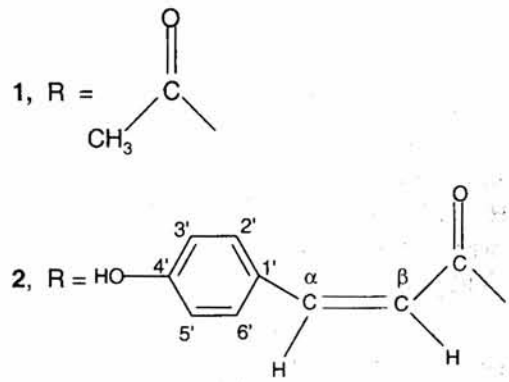


Table I—^{13}C- and ^{1}H- NMR spectral data of compound **1** in $CDCl_3$

Carbon[1]	DEPT	δ_C	δ_H	Carbon[1]	DEPT	δ_C	δ_H
1	CH_2	37.5	1.254	18	CH	48.9	1.302
2	CH_2	23.5	1.645	19	CH_2	36.7	1.347
3	CH	81.1	4.459(dd) (J=9.6,6.6Hz)	20	C	28.8	--
4	C	37.9	--	21	CH_2	33.2	1.362
5	CH	55.7	1.254	22	CH	81.1	3.641(t)
6	CH_2	18.7	1.387	23	CH_3	28.0	0.905(s)
7	CH_2	41.3	1.44	24	CH_3	16.6	0.951(s)
8	C	39.0	--	25	CH_3	15.5	0.876(s)
9	CH	49.3	1.318	26	CH_3	25.9	1.090(s)
10	C	37.9	--	27	CH_3	21.3	0.858(s)
11	CH_2	17.5	1.424	28	CH_3	29.9	0.819(s)
12	CH_2	33.7	1.399	29	CH_3	33.4	0.951(s)
13	C	37.6	--	30	CH_3	29.9	0.905(s)
14	C	158	--	-CO-CH_3	CH_3	21.3	2.043(s)
15	CH	117	5.532(dd) (J=8.1,3Hz)	-CO_2-	C	170.9	--
16	CH_2	37.7	1.677	-O-H	--	--	5.122(br)
17	C	35.8	--				

[1]Numbering as shown in structure of compound **1**

at m/z = 451 was generated as [M^+-(H_2O + -CH_3)] and m/z = 407 as [M^+ - (H_2O + -OAc)]. The ion at m/z = 344 was formed by a retro Diels-Alder fragmentation[8], a characteristic fragmentation of pentacyclic triterpenoids. This supported the proposed structure of lower part of the molecule and position of the double bond at C14-C15. The other prominent peak at m/z = 44 was due to acetyl moiety. On the basis of the foregoing discussion, the compound **1** was identified as 22-hydroxytaraxeryl acetate.

Compound **2** was obtained from the petroleum ether-ethyl acetate mixture (17:3). The compound responded positive colour reactions[3] with LB reagent (pink), VS reagent (blue), AS reagent (blue-violet) and 20% H_2SO_4 (pink). Also, it gave blue colouration with neutral ferric chloride (phenolic). It decolourised the pink colour of Baeyer's reagent indicating unsaturation in the molecule.

The IR spectrum of the compound **2** displayed a characteristic absorption at 1697 cm^{-1} indicating α,β-unsaturated ester carbonyl group, and 3700-3300 cm^{-1} for hydroxyl group in the compound.

The ^{1}H- and ^{13}C-NMR and DEPT-90° and 135° spectral data are similar to those reported for *cis-p*-hydroxycinnamoyl ester of taraxerol[5] with additional signals indicating the presence of a hydroxyl function and exhibited apentacyclic triterpenoid pattern as in compound **1**.

Inspection of the aromatic and olefinic region of the ^{1}H- and ^{13}C-NMR spectral data of the compound suggest a *para* disubstituted benzene ring and a *cis*-disubstituted olefinic group. The well resolved ^{1}H NMR signals at δ 7.64 (2H, d, J = 8.4 Hz) and 6.80 (2H, d, J = 8.1 Hz) were ascribed to 2', 6' and 3', 5' protons of the phenyl ring in the ester part

respectively. The ^{13}C NMR signals at 132.4 ppm and 115 ppm were attributed to 2', 6' and 3' 5' carbon atoms of the phenyl ring in the ester part.

The ^{1}H NMR signals at 6.83 (d, J = 13.2 Hz) and 5.83 (d, J = 12.9 Hz) were assigned to α- and β-olefinic H atoms of the cinnamoyl ester functionality. From the ^{1}H NMR signal values and coupling constants, it was concluded that the olefin was *cis* disubstituted (**Table II**).

In the mass spectrum, the peaks obtained were quite consistent with the proposed structure of the compound **2** and similar in all respect to that of compound **1**. The ion peak at m/z = 147 and 148 were due to the fragments of the cinnamoyl moiety.

On the basis of spectral data and chemical reactions, the compound **2** was characterized as 22-hydroxytaraxeryl-*cis*-p-coumarate.

Experimental Section

Melting points were determined using Toshniwal capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FTIR 8101-A spectrometer and the spectra were determined in KBr pellets. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity Plus–300 spectrometer using $CDCl_3$ a solvent with TMS as internal standard. The chemical shift are recorded in δ, ppm. The mass spectra were recorded on a Joel SX-102 mass spectrometer. The column chromatographic separation of the crude and semipurified extracts were carried out using silica gel (Acme, 100-200 mesh). The TLC plates were prepared using TLC grade silica gel-G (Acme). For preparative TLC, plates were prepared using Stahl apparatus.

Extraction and Isolation

The leaves of the plant were collected from Mattom Desam of Trichur District, Kerala, India and authenticated by Dr. A.K. Pradeep, Botany Department, Calicut University, Kerala. A voucher specimen (No. 70560) has been deposited in the Herbarium (CALI) of Botany Department, University of Calicut.

About 2 kg of the shade dried powdered leaves of the plant were extracted repeatedly using a Soxhlet extractor with pet. ether (60-80°C) for 36 hr. The combined extracts were then concentrated under reduced pressure using a rotary vacuum flash evaporator. It was then subjected to column chromatography and eluted with pet. ether followed by increasing proportions of ethyl acetate. Fractions eluting with pet. ether-ethyl acetate mixture (9:1 and 17:3, v/v) afforded the compounds **1** and **2**. The compounds were further purified by preparative TLC and recrystallised. The purity was checked on TLC in solvent systems of varying polarity.

Table II—^{1}H- and ^{13}C-NMR spectral data of **2** in $CDCl_3$

Carbon[1]	DEPT	δ_C	δ_H	Carbon[1]	DEPT	δ_C	δ_H
1	CH_2	37.4	1.254	21	CH_2	33.1	1.362
2	CH_2	23.5	1.645	22	CH	81.1	3.642 (t)
3	CH	81.1	4.459(dd) (J=9.6,6.6Hz)	23	CH_3	28	0.905 (s)
4	C	37.9	--	24	CH_3	16.6	0.951 (s)
5	CH	55.7	1.254	25	CH_3	15.5	0.876 (s)
6	CH_2	18.7	1.387	26	CH_3	25.95	1.090 (s)
7	CH_2	41.3	1.44	27	CH_3	21.3	0.858 (s)
8	C	39	--	28	CH_3	29.9	0.819 (s)
9	CH	49.2	1.318	29	CH_3	33.4	0.951 (s)
10	C	37.9	--	30	CH_3	30	0.905 (s)
11	CH_2	17.5	1.424	1'	C	127	--
12	CH_2	33.7	1.399	2', 6'	CH	132.4	7.645 (d)
13	C	37.6	--	3', 5'	CH	115	6.805 (d)
14	C	158	--	4'	C	157	--
15	CH	117	5.532(dd) (J=8.1,3.0Hz)	α	CH	143	6.836 (d)
16	CH_2	37.7	1.677	β	CH	117	5.83 (d)
17	C	35.8	--				
18	CH	48.8	1.302	$-CO_2-$	C	171.1	--
19	CH_2	36.7	1.347		Phenolic OH	--	3.934
20	C	28.8	--		Alcoholic OH	--	3.492

[1]Numbering as shown in structure of compound **2**

Compound **1**. $C_{32}H_{52}O_3$, colourless powder (100 mg), m.p. 250-252°C; IR (KBr): 3917-3265 (O-H str), 2922, 2852.5 (C-H str, sym and sym), 1724.2 (ester carbonyl str), 1649 (C=C str), 1467.7 cm^{-1} (C-H bend); MS *m/z* (rel int): 466 (M^+ - H_2O, 18), 451 (11), 407 (8), 391 (8), 344 (15), 343 (35), 203 (100), 44 (91); ^{13}C NMR (DEPT) and ^1H NMR ($CDCl_3$): (**Table I**).

Compound **2**. $C_{39}H_{56}O_4$, colourless, powder (200 mg), m.p. 245-247°C; IR (KBr): 3361.7 (O-H str), 2929.7, 2856.4 (C-H str, asym and sym), 1697.2 (α,β-unsaturated ester carbonyl), 1631.7 (C=C str), 1251.7 cm^{-1} (C-O-C str); MS *m/z* (rel int): 466 (22), 451 (18), 407 (14), 391 (15), 344 (12), 343 (60), 203 (100), 147 (40), 148 (50); ^{13}C NMR (DEPT) and ^1H NMR ($CDCl_3$): (**Table II**).

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