



A New Terpenoid 3R, 4R, 4aR, 5R, 6aS, 6bR, 12aR)-4a (Hydroxyl methyl)-2,2,6a,6b,7,8,8a,9,10,11,12,12a, 12b,13,14b-Icosahdropicene, 3,4,5,10-tetraol from *Scutellaria scandance*

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ABSTRACT

From ethanolic extract of *Scutellaria scandens* plant a 3R, 4R, 4aR, 5R, 6aS, 6bR, 12aR)-4a(hydroxyl methyl)- 2,2,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahdropicene, 3,4,5,10-tetraol terpenoid has been isolated and characterized by means of rigorous spectroscopic analysis including 2 D NMR measurements. This is new study in chemical analysis of *Scutellaria scandens*.

Keywords: *Scutellaria scandens*, 3R, 4R, 4aR, 5R, 6aS, 6bR, 12aR)-4a(hydroxyl methyl)-2,2,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahdropicene, 3,4,5,10-tetraol, antimicrobial activity, *Klebsiella pneumoniae* and *Mycobacterium smegmatis*.

INTRODUCTION

Material and method

Scutellaria scandens belong to family lamiaceae perrinnial erect shrub with actual 4-angled, glabresecent or hairy branched, leaves ovate-lanceolate flower, pale-yellow or nearly white interterminal. Occure in open places edges of fields and forest floor to 2500m altitude. Localy it is used in antivomating and antidisentry¹. *S.scandens* [leaves] Pinosylvin-3-O- β -D-glucopyranoside and 3,5-dihydroxytras-stilbene-2-carboxylic acid. 2, 4 dihydroxy-phenylethy 6-O-sinapoly- β -D glucopyranoside and 4-methoxy carbonyl methyl

phenyl 6-sinapoly². *S.prostrata* [Root] 5-6-2'-6'-tratrahydroxy-7-8-dimethoxyflavone, 5, 6, 2' trihydroxy-7-8-6' trimethoxy, 5-7-2' trihydroxy 8-methoxy flavone, 7'-O- β -D-glucopyranoside, 2-ethyl-1-O- β -D-glucopyranoside³. *S.indica* [Root] 2'' dihydroxy-7-8-6'-trimethoxy flavone, 5-2'' dihydroxy-6-7-6'-trimethoxy flavonone, 5-7 dihydroxy-6-7-6' trimethoxy flavonone, 5-7-dihydroxy-8-2'-dimethylflavanone, rivularia-5-2'- trihydroxy-7-8-dimethylflavone, scutevurin-5-7-4' trihydroxy-8-methyl flavone⁴. The whole plant of *Scutellaria scandens* were collected from Bacchehar District. Chamoli Utrakhnad in the month of October and identified by Department Botany, P.G. College

Gopeshwar where vaucher specimen was deposited. The air dried whole plant (3kg) was exhaustively extracted with 90% aqueous EtOH for 72 hours. The ethanol extract was concentrated to dryness. The dry ethanolic extract was chromatographic over silica-gel using Methanol Chloroform (20:80) as elution solvent which afforded the compound.

RESULTS

It was crystallized from MeOH as colourless, amorphous solid, M.P 140°C compound was obtained as colourless, amorphous solid from

MeOH was found to have element analysis found values C=73.42%, H=10.15%, required values for C₃₀H₅₀O₅: C=73.47%, H=10.20%; molecular weight 490 as presumed by the presence of molecular ion at m/z 490 in the EI-positive mass spectra showed peaks at m/z 490, 475, 436, 282, 264, 251, 246, 237, 207, 165, 149. Element analysis of compound corresponded to the molecular formula C₃₀H₅₀O₅ showing six double bond equivalent (DBE) in the molecule.

The molecular formula corresponded to triterpenoids and since it have six DBE, it must have pentacyclic skeleton with either one double bond

Table 1:

C/H No.	°C	Multiplicity (DEPT)	¹ H (J in Hz)	HMBC Correlation (H-C)
1.	39.1	CH ₂	1.06, m; 1.62 m	C-10, C-25
2	28.2	CH ₂	1.88-1.95, m	C-3, C-1
3	78.1	CH	3.47 dd (10.2, 6.3)	C-23, C-24
4	39.4	C	-	-
5	55.8	CH	0.90, m	C-6
6	18.8	CH ₂	1.60, m	C-5, C-7, C-8, C-10
7	33.3	CH ₂	1.38, m; 1.68 m	C-6, C-8
8	40.1	C	-	-
9	47.2	CH	1.82, m	C-10, C-11, C-25, C-26
10	37.3	C	-	-
11	23.9	CH ₂	1.90-2.00, m	C-8, C-12, C-13
12	123.5	CH	5.42, dd, (3.3, 3.9)	C-9, C-14
13	144.0	C	-	-
14	42.0	C	-	-
15	34.3	CH ₂	1.70, m; 2.13, m	C-8, C-14, C-16, C-27
16	67.9	CH	5.04, m	-
17	47.3	C	-	-
18	41.2	CH	2.82, m	C-12, C-13, C-16, C-17, C-19
19	48.3	CH ₂	1.42, m; 3.05, m	C-18, C-20, C-29, C-30
20	36.4	C	-	-
21	78.7	CH	4.79, d (4.8)	C-20, C-22, C-30
22	77.3	CH	4.62, d (4.8)	C-16, C-17, C-21, C-28
23	28.7	CH ₃	1.24, s	C-2, C-3, C-4, C-5, C-24
24	16.6	CH ₃	1.06, s	C-2, C-3, C-4, C-5, C-23
25	15.8	CH ₃	0.97, s	C-1, C-10
26	17.0	CH ₃	0.98, s	C-8, C-14
27	27.4	CH ₃	1.86, s	C-8, C-9, C-14
28	68.4	CH ₂	3.74, d (14.4); 4.03, d (14.4)	C-16, C-17, C-18, C-22
29	30.6	CH ₃	1.34, s	C-19, C-20, C-21, C-30
30	19.5	CH ₃	1.40, s	C-19, C-20, C-21, C-29

or a carbonyl group. The IR (Vmax KBr): cm^{-1} spectrum at 3400-3280, 2900, 1460, 1380, 1045, 1010 etc. IR spectrum contained absorption band due to hydroxy group at 3400-3280 cm^{-1} and 2900 cm^{-1} (C-H stretching) of methyl functions. The UV spectrum showed absorption maxima at 217 nm, the characteristic of triterpenoid having double bond at C12-C-13 carbon atom (5)

In $^1\text{H-NMR}$ spectra of compound revealed the presence of seven tertiary methyl groups (0.97, 0.98, 1.06, 1.24, 1.34, 1.40, 1.86), one tri-substituted double bond (5.42), and an oxymethylene group at 3.74, d ($J=14$ Hz) and 4.03, d ($J=14$ Hz). In addition there were four oxymethine protons at 3.47 (dd, $J=10.2, 6.3$ Hz, H-3), 5.04 (1H, m, H-16), 4.79 (1H, d, $J=4.8$ Hz, H-21) and 4.62 (1H, d, $J=4.8$ Hz, H-22), seven methylene proton signals and five methine proton signals were observed. Since there were no signals in the aromatic region, this indicated the pentacyclic triterpenoid skeleton of the molecule (5).

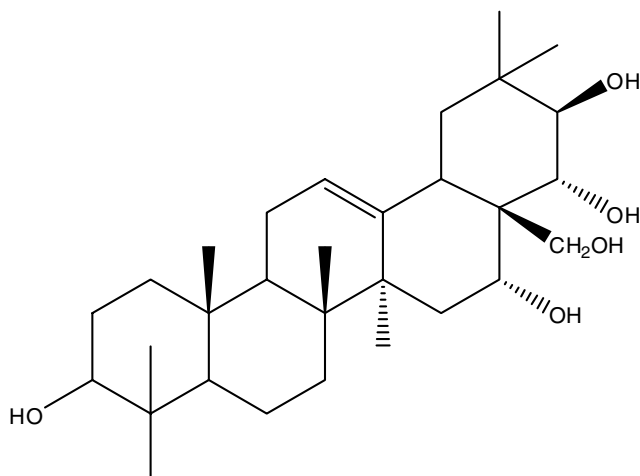
The $^{13}\text{C-NMR}$ spectrum showed presence of 30 carbon atoms in the molecule. The multiplicity of carbon resonances were determined by the DEPT spectrum which confirmed the presence of seven quaternary carbon, seven methyl carbon, eight methylene carbon atoms in the molecules. The assignment of carbon atom and proton attached with them were confirmed by the HMQC spectrum which showed one bond heteronuclear coupling between ^1H - and ^{13}C - nuclei. The ^{13}C -chemical shifts of carbon atoms at 28.7 (C-23), 16.6 (C-24), 15.8 (C-25), 17.0 (C-26), 27.4 (C-27), 30.6 (C-29) and 19.5 (C-30) confirmed presence of seven methyl group. The downfield chemical shift of oxygenated carbon atoms at 78.1, 67.9, 78.8, 77.3 and 68.4 indicate the presence of five hydroxyl group in the molecule. The ^{13}C - chemical shift of carbon atoms at 123.5 (C-12) and 144.0 (C-13) confirmed presence of double bond in the molecule.

The fragment ion peak at m/z 282, $[\text{C}_{16}\text{H}_{26}\text{O}_4]^+$, 251 $[\text{C}_{15}\text{H}_{23}\text{O}_3]^+$ 237 $[\text{C}_{14}\text{H}_{21}\text{O}_3]^+$ derived from ring D, E and at m/z 207 $[\text{C}_{14}\text{H}_{13}\text{O}]^+$ derived from ring A, B via Retro-Diels- Alder fission in the EI- mass spectrum indicate that compound possess four hydroxyl groups in the D,E ring and

one hydroxyl group in A,B-ring on the olean-12 ene skeleton [5-8]. The ^1H - chemical shift of a proton at 5.42 appear as dd, ($J=3.3, 3.9$ Hz) due to typical H-12 proton of a tri-substituted double bond and a multiplet at 2.82, due H-18 proton confirmed the olean-12-ene skeleton of the molecules⁵.

The $^1\text{H-}^{13}\text{C}$ heteronuclear multiple bond correlation (HMBC) spectrum which showed long range correlation between a given proton and carbon atoms around it determined the absolute structure of the molecule. The methyl group at 1.24 (H-23) showed $^2\text{J}_{\text{CH}}$ correlation with C-4, $^3\text{J}_{\text{CH}}$

Correlation with C-24, C-3 and C-5 and $^4\text{J}_{\text{CH}}$ correlation with C-2 while the methyl singlet at 1.06 (H-24) showed $^2\text{J}_{\text{CH}}$ correlation with C-4, $^3\text{J}_{\text{CH}}$ correlation with C-23, C-3 and C-5 and 4JCH correlation with C-2. In addition, the methine proton at 3.77 (H-3) showed $^3\text{J}_{\text{CH}}$ correlation with C-23 and C-24 couple with the ^1H and ^{13}C chemical shift of proton and carbon assigned for position 4, 23 and 24 confirmed that gem-methyl groups are attached at C-4 carbon atom. Similarly the methyl group at 1.34 (H-29) showed $^2\text{J}_{\text{CH}}$ correlation with C-20 and $^3\text{J}_{\text{CH}}$ and correlation with C-19, C-21 and C-29 showed that gem-methyl groups attached with C-20 and $^3\text{J}_{\text{CH}}$ carbon atoms. In addition to this the methylene proton at 1.42 (H-19) showed $^3\text{J}_{\text{CH}}$ correlation with C-29, 30 and $^2\text{J}_{\text{CH}}$ correlation with C-18 and C-20; and the methine proton attached with oxygen bearing carbon at 4.79 showed $^3\text{J}_{\text{CH}}$ correlation with C-30 and $^2\text{J}_{\text{CH}}$ correlation with C-20 and C-22 confirmed the location of gem-methyl group at C-20 position. The methyl protons at 0.97 (H-25), showed $^2\text{J}_{\text{CH}}$ correlation with C-10 and $^3\text{J}_{\text{CH}}$ correlation with C-1 only indicated that methyl function was attached at C-10, which was confirmed by $^3\text{J}_{\text{CH}}$ correlation of methylene protons (1.06, m; 1.62 m, H-1) with C-25 and $^3\text{J}_{\text{CH}}$ correlation of methine proton (at 1.82, H-9) with C-25. The methyl proton 0.98 (H-26) showed $^2\text{J}_{\text{CH}}$ correlation with C-8 and $^3\text{J}_{\text{CH}}$ correlation with C-14 while the methyl proton at 1.86 (H-27) showed $^2\text{J}_{\text{CH}}$ correlation with C-14 and $^3\text{J}_{\text{CH}}$ correlation with C-8 determined their location at position C-8 and C-14 respectively. The oxy-methylene protons (H-28) at 3.74, d ($J=14.4$ Hz) 4.03, d ($J=14.4$ Hz) showed $^2\text{J}_{\text{CH}}$ correlation with C-17 and $^3\text{J}_{\text{CH}}$ correlation with C-22 and C-16 and C-18 confirmed that a $-\text{CH}_2\text{OH}$ function was



(3*R*,4*R*,4*aR*,5*R*,6*aS*,6*bR*,12*aR*)-4-(hydroxymethyl)-2,2,6*a*,6*b*,9,9,12*a*-heptamethyl-1,2,3,4,4*a*,5,6,6*a*,6*b*,7,8,8*a*,9,10,11,12,12*a*,12*b*,13,14*b*-icosahydricene-3,4,5,10-tetraol

attached at C-17 position⁹, The large coupling constant (14.4 Hz) determined that the beta-orientation of these methylene proton which in turn determined that the alpha-orientation of -CH₂OH function. The J-values of H-3, H-16, H-21 and H-22 proton signals and comparison of their ¹H and ¹³C-chemical data with the related compound determined that these signals are oriented axially

and thus determined the 3beta, 16alpha, 21alpha-orientation⁵.

On the basis of above discussed spectral data compound was characterized as methyl olen-12-ene-3beta, 16alpha, 21beta,22alpha,28-pentaol.

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