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Isolation of keto alcohol and triterpenes from tubers of *Cyperus rotundus* Linn.

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ABSTRACT

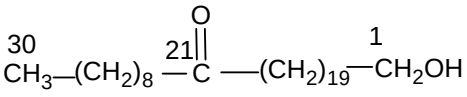
Phytochemical investigation of the methanolic extract of the tubers of Cyperus rotundus L. (Cyperaceae) led to the isolation of one new keto alcohol characterized as n-tricont-1-ol-21-one, two new triterpenic glucosides 18α-H-urs-12-en-3 β-ol- β-D-glucuronopyranoside (18-epi-α-amyrin glucuronoside) and 3 β-hydroxyolean-12-en-28-oic acid α-D-arabinofuranoside (oleanolic acid arabinoside) along with the known compounds Urs-12-en-3β–ol-3-β-D-glucopyranoside (α -Amyrin glucopyranoside), Olean-12-en-3β–ol-3-β-D- glucopyranoside (β -Amyrin glucopyranoside). The structures of all these phytoconstituents have been elucidated on the basis of ^{1}H NMR, ^{13}C NMR, and FAB MS spectral data analysis and chemical reactions.

Keywords: *Cyperus rotundus*, ricipentatriacontanol, keto alcohol, 18-epi-α-amyrin glucuronoside, oleanolic acid arabinoside.

INTRODUCTION

Cyperus rotundus Linn. (Family Cyperaceae), a perennial weed, found throughout India up to an elevation of 1, 829m. The dried rhizome is used in loss of appetite, indigestion, excessive thirst, pyrexia, cough, vomiting, lacteal disorders, diarrhea, rheumatoid arthritis and worm infestation, The tubers also posses anti-inflammatory and hypotensive properties [1-4].

The tubers yielded terpenoidal and flavonoidal constituents:sitosterol, oleonolic acid-3-O-neohesperidoside [5]; flavonol glycoside, rhamnetin 3-O-rhamnosyl rhamnopyranoside [6]; caryophyllene, caryophyllene-6, 7-oxide, caryophylla-6-one [7]; unidentified terpenoids [8]; sesquiterpene 10,12- peroxycalamenene, patchoulenone, caryophyllene α-oxide, 4,7-dimethyl-1-tetralone [9]; 4α, 5α-oxidoeudesm-11-en-3 α-ol [10]; (-)-isorotundene, (-)-cypera-2, 4 (15)-diene, (-)- norrotundene and (+)- cyperadione [11] and alkaloid rotundines-A, B and C [12]. The present paper describes the isolation and characterization of one new keto alcohol n-tricont-1-ol-21-one (**1**) and two new triterpenic glycosides 18α-H-urs-12-en-3 β-ol- β-D-glucuronopyranoside (18-epi-α-amyrin glucuronoside) (**2**) and 3 β-hydroxyolean-12-en-28-oic acid α-D-arabinofuranoside (oleanolic acid arabinoside) (**3**) as new constituents and two known phytoconstituents Urs-12-en-3β–ol-3-β-D-glucopyranoside (α -Amyrin glucopyranoside) (**4**), Olean-12-en-3β–ol-3-β-D- glucopyranoside (β -Amyrin glucopyranoside) (**5**) from the tuber of *Cyperus rotundus* Linn.



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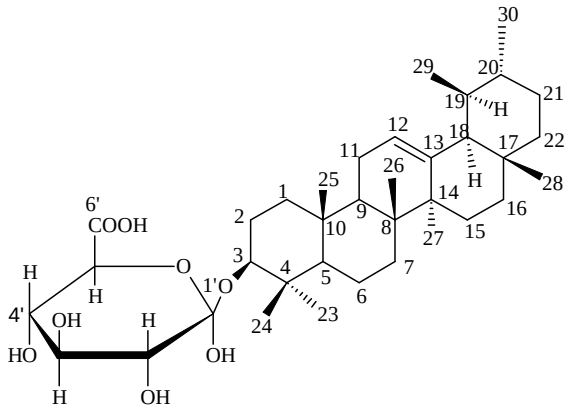

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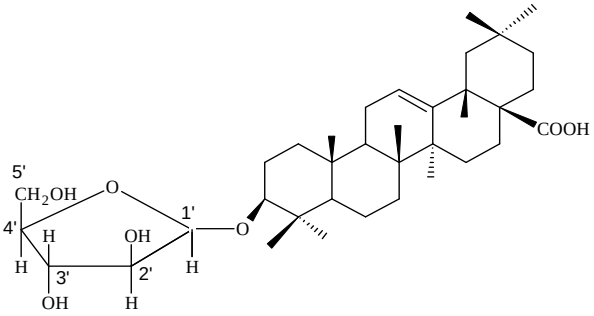

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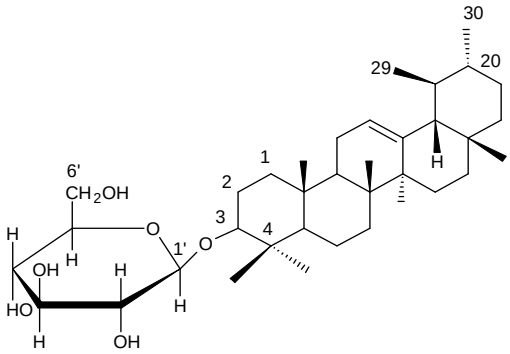

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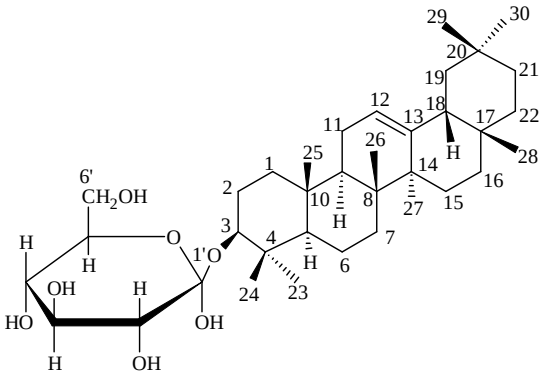

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RESULTS AND DISCUSSION

Compound **1**, a ketoalcohol, was obtained as a colourless crystalline mass from chloroform- methanol (99:1) eluants. It formed 2,4-dinitrophenyl hydrazine derivative indicating the presence of a carbonyl group in the molecule. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3437 cm^{-1}), carbonyl group (1703 cm^{-1}) and long aliphatic chain (755 cm^{-1}). The mass spectrum of **1** exhibited a molecular ion peak at m/z 452 corresponding to a molecular formula of a ketoalcohol, C_{30} $H_{60}O_2$. It had one degree of double bond equivalent, which was adjusted in the carbonyl group. The ion fragments arising at m/z 127 $[CH_3 (CH_2)_8]^+$, 325 [M-127, CO $(CH_2)_{19}$ CH_2 $OH]^+$, 155 $[CH_3 (CH_2)_8 CO]^+$, 297 [M-155, $(CH_2)_{19}$ $CH_2OH]^+$ suggested the existence of the keto group at C-21. The ^1H NMR spectrum of **3** showed a two- proton broad signal at δ 3.30 assigned to oxygenate methylene H_2-1 protons. Two multiplets at δ 2.14 and 2.01, both integrated for two protons each, were ascribed correspondingly to C-20 and C-22 methylene protons adjacent to the keto carbon. A three- proton triplet at δ 0.84

(*J*=6.5 Hz) was accounted to C-30 primary methyl protons. The remaining methylene appeared between δ 1.63-1.03. The ^{13}C NMR spectrum of **1** displayed signals for carbonyl carbon at δ 207.13 (C-21), hydroxymethylene carbon at δ 60.61 (C-1), methylene carbons in the range of δ 33.46-20.40 and methyl carbon at δ 14.36 (C-30). The absence of any ^{1}H NMR signal beyond δ 3.30 and ^{13}C NMR signal betweenδ 207.13-60.61 supported the saturated nature of the molecule. The HMBC spectrum of **1** exhibited correlations of C-1 with H_2-2; C-21 with H_2-20 and H_2-22; and C-30 with H_2-29. On the basis of these evidences the structure of **1** has been formulated as n-triacont-1-ol-21-one. This is a new keto alcohol isolated from a plant or synthetic source for the first time.

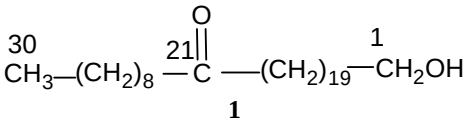

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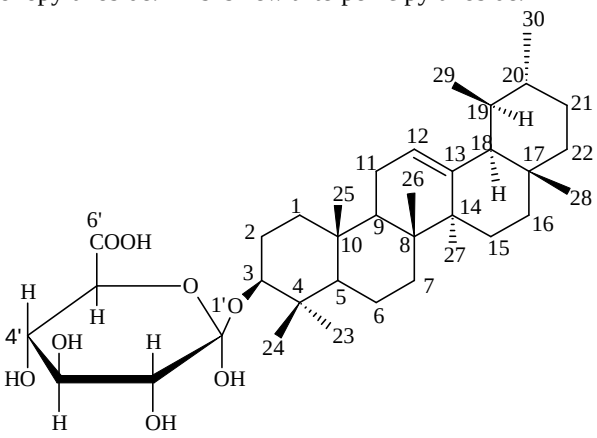
Table 1. ^{1}H NMR and ^{13}C NMR Spectral Values Of 18-Epi-A-Amyrin Glucuronoside (2)

Position	^{1}H NMR		^{13}C NMR
	α (Alpha)	β (Beta)	
1	1.90 dddd (3.2, 11.2, 7.2, 2.8)	1.35 dddd (11.2, 8, 5.6, 3.9)	38.87
2	1.87 m	1.66 m	27.24
3	3.31 dd (3.7, 8.1)	-	78.32
4	-	-	38.75
5	1.04 m	-	55.23
6	1.50 m	1.01 m	18.33
7	1.07 ddd (12.5, 8.8, 4.8)	1.32 ddd (8.0, 5.6, 8.8)	33.07
8	-	-	39.02
9	1.45 dd (9.2, 4.0)	-	47.31
10	-	-	38.68
11	1.62 dd (4, 8.8)	1.70 dd (8.8, 2.8)	23.22
12	5.30 m	-	125.20
13	-	-	138.33
14	-	-	52.72
15	1.33 ddd (11.6, 8, 5.6)	1.61 ddd (4, 8.8, 9.2)	27.96
16	0.77 dd (9.6, 5, 5.3)	1.45 dd (3.6, 4.0)	23.56
17	-	-	36.76
18	-	2.71 d (6.3)	42.01
19	2.27 m	-	42.85
20	-	1.11 m	30.69
21	1.25 ddd (5.6, 7.6, 14.0)	1.28 ddd (8, 5.6, 14. 0)	29.58
22	1.47 dd (3.6, 9.2)	1.45 dd (3.6, 4.21)	36.91
23	0.86 brs	-	28.34
24	0.96 brs	-	15.97
25	0.91 brs	-	15.49
26	0.75 brs	-	17.17
27	0.94 brs	-	17.13
28	1.04 brs	-	21.30
29	0.85 d (6.4)	-	24.21
30	0.83 d (6.0)	-	31.80
1'	4.67 d (7.0)	--	108.26
2'	3.31 brs	--	73.47
3'	2.27 brs	--	67.35
4'	2.32 brs	--	71.73
5'	3.77 brs	--	76.59
6'	---	--	179.64

Coupling constants in Hertz are given in parenthesis.

Compound **2**, α- amyrin glucuronoside, was obtained as a colourless amorphous powder from chloroform: methanol (97:3) eluants. It responded positively to Liebermann-Burchardt test for triterpenoids and tests of glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxy groups (3350 cm^{-1}), carboxylic group (1691 cm^{-1}) and unsaturation (1645 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 602 corresponding to molecular formula $C_{36}H_{58}O_7$. The formula exhibited the presence of eight double equivalents, five of which were adjusted in a pentacyclic carbon framework and one each in a vinylic linkage, sugar moiety and a carboxylic group. The mass

spectrum displayed characteristic ion fragments at *m/z* 425 $[M-C_6H_9O_6]^+$ and at m/z 207 and 219 generated due to retro-Diels Alder fragmentation suggesting Δ^{12} olefinic linkage in ring C [13, 14] and the presence of glucuronic acid moeity in the compound. The ion fragments generated at *m/z* 189 $[207-H_2O]^+$, 174 $[189-Me]^+$, 159 $[174-Me]^+$, 144 $[159-Me]^+$, 129 $[144-Me]^+$, 192 $[207-Me]^+$, 177 $[192-Me]^+$, 162 $[177-Me]^+$, and 147 $[162-Me]^+$ indicated the location of four methyl groups and a carbonyl group in the ring A/B which was placed at C-3 on the basis of biogenetic considerations. The ion peaks arising at *m/z* 203 $[219-Me]^+$, 188 $[203-Me]^+$, 173 $[188-Me]^+$ and 158 $[173-Me]^+$ supported the existence of other methyl groups the carboxylic group in the ring D/E. The ^{1}H NMR spectrum of **2** displayed a downfield multiplet centered at δ 5.30 that was assigned to H-12 vinylic proton. A doublet at δ 4.67 (*J*= 7.0 Hz) was ascribed to H-1' anomeric proton while as other sugar protons appeared as four one-proton multiplets at δ 3.77-2.27. A one-proton double-doublet at δ 3.31 was assigned to carbinol proton that was placed at C-3 in α-orientation on the basis of its coupling constant (*J* = 3.7, 8.1 Hz). Another one-proton doublet at δ 2.71 (*J* = 6.3 Hz) was assigned to H-18α proton. The remaining methylene protons resonated in the range from 1.04-2.27. Six three-proton broad signals at δ 0.86 (Me-23), 0.96 (Me-24), 0.91 (Me-25), 0.75 (Me-26), 0.94 (Me-27) and 1.07 (Me-28) and two three-proton doublets at δ 0.85 (*J* = 6.4 Hz) and δ 0.83 (*J*= 6.0 Hz) were ascribed to Me-29 and Me-30 methyl protons of ursene type compound, all located on the saturated carbons. The ^{13}C NMR spectrum of **2** displayed signals for thirty six carbons. The important signals appeared at δ 179.64 for carboxylic carbon (C-6'), δ 138.33 and 125.20 for vinylic carbons (C-13 and C-12), at δ 108.26 for anomeric carbon (C-1') and δ 78.32 for carbinol carbon (C-3). The remaining sugar carbons appeared between δ 76.59-67.35. The methyl carbons resonated from δ 15.49 to 28.34. The assignments of the carbon chemical shift of **2** were made by comparison with δ values of corresponding carbon atom of urs-12-enes [14, 15]. The HMBC spectrum of **2** exhibited interactions of C-3 with H_2-2, H_3-23 and H-1', C-13 with H-12 and H-18; and C-6' with H-5'. Acid hydrolysis of **2** yielded α- amyrin and D-glucuronic acid. On the basis of spectral data analysis and chemical reactions, the structure of **2** was elucidated as urs-12-en-3 β-ol- β-D- glucuronopyranoside. This is new triterpenic pyranoside.



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Compound **3**, named oleanolic acid arabinoside, was obtained as a colourless crystalline mass from chloroform: methanol (19:1) eluants. It responded positively to Liebermann Burchardt test for triterpenoids and tests of glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxy groups (3424 cm^{-1}), carboxylic group (1705 cm^{-1}) and unsaturation (1646 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 588 corresponding to molecular formula $C_{35}H_{56}O_7$. The formula showed the presence of eight double equivalents, five of which were adjusted in a pentacyclic carbon framework and one each in a vinylic linkage, sugar moiety and one in a carboxylic group. The mass spectrum displayed characteristic ion fragments at *m/z* 456 $[M-C_5H_9O_4]^+$, and at m/z 207 and 248 generated due to retro-Diels Alder fragmentation suggesting Δ^{12}-olefinic linkage in ring C [13, 14] and sugar unit in the molecule. The ion fragments generated at *m/z* 192 $[207-Me]^+$, 177 $[192-Me]^+$, 162 $[177-Me]^+$, 147 $[162-Me]^+$, 189 $[207-H_2O]^+$, 174 $[189-Me]^+$, 159 $[174-Me]^+$, 144 $[159-Me]^+$, and 129 $[144-Me]^+$ indicated the presence of carbinol carbon in the ring A/B, which was placed at C-3 on the basis of biogenetic analogy. The ion peaks formed at *m/z* 411 $[456-COOH]^+$, 203 $[248-COOH]^+$, 188 $[203-Me]^+$, 173 $[188-Me]^+$ and 158 $[173-Me]^+$ supported the existence of the carboxylic group in the ring D/E. The ^{1}H NMR spectrum of **3** displayed a downfield one-proton doublet at δ 5.33 (*J*= 3.2 Hz) that was assigned to H-12 vinylic proton. The α-oriented anomeric proton H-1' appeared as one-proton doublet at δ 5.01 (*J*= 5.7 Hz). The remaining sugar protons appeared as four multiplets

at δ 4.79 (1H), 4.51 (1H), 4.51 (1H), 4.40 (1H) and 3.36 (2H) assigned correspondingly to H-2', H-3', H-4' and H_2-5' protons. A one-proton double-doublet at δ 3.16 was assigned to carbinol proton that was placed at C-3 in α-orientation on the basis of its coupling constant (*J* = 4.1, 9.3 Hz). A one-proton double doublet at δ 2.62 (*J* = 7.2, 3.6 Hz) was assigned to H-18β proton. The methylene protons resonated in the range from 1.01-1.67. Seven three-proton broad signals at δ 0.87 (Me-23), 0.96 (Me-24), 0.91 (Me-25), 0.75 (Me-26), 1.07 (Me-27), 0.94 (Me-29) and 0.85 (Me-30) were ascribed to tertiary methyl protons of oleanene-type compound. The presence of methyl signals in the region δ 1.07-0.75 indicated that all these functionalities were located on the saturated carbons. The ^{13}C NMR spectrum of **3** displayed signals at δ 179.54 for carboxylic carbon (C-28), δ 138.35 and 125.18 for vinylic carbons (C-13, C-12), 109.78 for anomeric carbon (C-1') and δ 78.52 for carbinol carbon (C-3). The methyl carbon resonated from δ 15.52-28.38. The remaining glucose carbons appeared between δ 72.11 to 61.5. The assignments of the carbon chemical shift of triterpenic skeleton were made by comparison with δ values of corresponding carbon atoms of urs-12-enes [14,15]. The HMBC spectrum of **3** showed correlations of C-3 with H_2-2; H_3-23 and H-1'; C-13 with H-12 and H-18; C-28 with H-8, H_2-16 and H_2-22 and C-4' with H-3' and H_2-5'. Acid hydrolysis of **3** yielded oleanolic acid and D-arabinose.

Table 2. ^{1}H NMR And ^{13}C NMR Spectral Values Of Oleanolic Acid Arabinoside (3)

Position	^{1}H NMR		^{13}C NMR
	α (Alpha)	β (Beta)	
1	1.90 ddd (3.2, 7.2, 11.6)	1.32 ddd (48, 9.2, 3.2)	38.68
2	1.88 m	1.63 m	27.16
3	3.16 dd (4.1, 9.3)	-	78.52
4	-	-	38.88
5	1.07 m	-	52.73
6	1.50 m	1.01 m	18.34
7	1.04 m	1.01 m	33.03
8	-	-	39.02
9	1.47 dd (9.6, 4.4)	-	47.46
10	-	-	38.76
11	1.63 dd (4.4, 8.8)	1.67 dd (3.6, 6.4)	23.56
12	5.33 d (3.2)	-	125.18
13	-	-	138.35
14	-	-	55.24
15	1.34 ddd (10.4, 9.2, 4.8)	1.61 ddd (4.8, 6.4, 3.6)	27.98
16	0.77 ddd (8.8, 10.8, 9.2)	1.45 ddd (3.6, 4.4, 9.6)	23.67
17	-	-	36.76
18	-	2.62 dd (7.2, 3.6)	42.08
19	1.58 d (7.2)	1.55 d (3.6)	40.53
20	-	1.11 m	30.70
21	1.25 m	1.30 m	29.57
22	1.28 dd (9.2, 4.8)	1.41 dd (9.2, 10.4)	36.92
23	0.87 brs	-	28.38
24	0.96 brs	-	16.02
25	0.91 brs	-	15.52
26	0.75 brs	-	17.18
27	1.07 brs	-	17.15
28	--	-	179.54
29	0.94 brs	-	31.5
30	0.85 brs	-	24.21
1'	5.01 d (*J*= 5.7)	--	109.78
2'	4.79 m	--	72.11
3'	4.51 m	--	71.51
4'	4.40 m	--	68.4
5'	3.36 m	--	61.5

Coupling constants in Hertz are given in parenthesis.

basis of above discussion the structure of **3**was elucidated as 18 β H-3 β-hydroxyolean-12-en-28-oic acid α-D-arabinofuranoside. This is new triterpenic arabinoside.

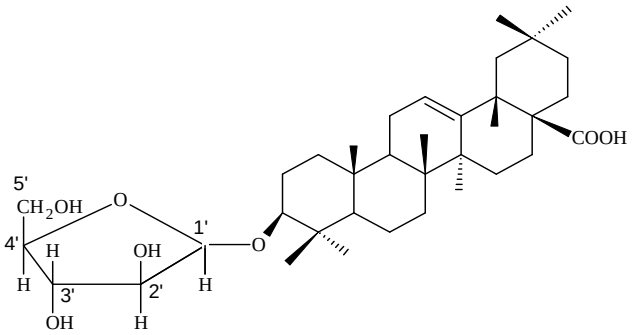

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Table 3. ^{1}H NMR And ^{13}C NMR Spectral Values Of A-Amyrin Glucopyranoside (4)

Position	^{1}H NMR		^{13}C NMR
	α (Alpha)	β (Beta)	
1	1.99 ddd (8.8, 10.0, 3.2)	1.35 ddd (3.2, 13.6, 13.6)	38.73
2	1.88 m	1.60 m	27.15
3	3.39 dd (5.1, 9.5)	-	78.39
4	-	-	38.84
5	1.28 m	-	55.20
6	1.49 m	1.30 m	18.30
7	1.32 m	1.38 m	33.00
8	-	-	39.01
9	1.49 dd (9.5, 4.1)	-	47.44
10	-	-	38.65
11	1.66 dd (7.2, 8.0)	1.88 m	23.21
12	5.21 d (3.5)	-	126.02
13	-	-	138.30
14	-	-	52.69
15	1.38 m	1.60 ddd (7.2, 8.0, 8.4)	27.96
16	1.30 m	1.47 dd (3.6, 4.8)	23.16
17	-	-	36.75
18	2.01 d (10.8)	-	41.99
19	2.33 m	-	41.99
20	-	1.12 m	30.68
21	1.28 m	1.30 m	29.57
22	1.50 dd (4.3, 9.1)	1.46 dd (3.6, 4.3)	36.89
23	0.96 brs	-	28.30
24	0.95 brs	-	15.94
25	0.69 brs	-	15.48
26	0.59 brs	-	24.10
27	1.11 brs	-	17.14
28	1.32 brs	-	21.29
29	0.81d (6.1)	-	32.1
30	0.83 d (6.0)	-	31.81
1'	4.73 d (7.2)	--	104.5
2'	3.48 brs	--	71.5
3'	3.48 brs	--	72.5
4'	3.48 brs	--	69.5
5'	4.04 brs	--	62.5
6'	3.48 brs	--	76.90

Coupling constants in Hertz are given in parenthesis.

Table 4. ^{1}H NMR And ^{13}C NMR Spectral Values Of B-Amyrin Glucopyranoside (5)

Position	^{1}H NMR		^{13}C NMR
	α (Alpha)	β (Beta)	
1	1.99 ddd (8.0, 9.6, 4.0)	1.32 ddd (8.3, 6.0, 11.2)	38.76
2	1.92 m	1.61 m	27.25
3	3.14 dd (5.6, 8.1)	-	78.47
4	-	-	38.87
5	1.28 m	-	55.22
6	1.52 m	1.38 m	18.33
7	1.32 m	1.41 m	33.02
8	-	-	39.01
9	1.58 dd (2.0, 9.2)	-	47.45
10	-	-	38.67
11	1.68 dd (9.2, 3.6)	1.86 dd (4.8, 3.6)	23.22
12	5.25 m	-	125.18
13	-	-	138.34
14	-	-	52.71
15	1.50 dd (9.2, 3.6,)	1.61 dd (3.2, 4.0)	27.97
16	1.30 dd (16, 8.4)	1.54 m	23.57
17	-	-	47.42
18	-	2.78 dd (11.4, 6.3)	42.01
19	1.41 m	1.38 m	39.50
20	-	-	30.69
21	1.58 dd (2.0, 9.2)	1.88 dd (4.8, 3.6)	29.58
22	1.66 dd (9.2, 3.6)	1.47 dd (3.6, 4.0)	36.91
23	0.85 brs	-	28.37
24	0.96 brs	-	16.02
25	0.90 brs	-	15.51
26	0.75 brs	-	17.17
27	1.07 brs	-	21.33
28	0.70 brs	-	33.51
29	0.94 brs	-	31.82
30	0.81 brs	-	24.20
1'	4.47 d (7.1)	--	104.11
2'	4.24 brs	--	73.80
3'	3.14 brs	--	71.70
4'	3.40 d (6.0)	--	66.51
5'	4.33 brs	--	63.35
6'	3.27 d (6.0)	3.27 d (6.0)	76.40

Coupling constants in hertz are given in parenthesis.

MATERIALS AND METHODS

3. Experimental

3.1 General Experimental Procedure

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were run by Bruker spectrospin NMR instrument in $CDCl_3$, using TMS as internal standard. FAB MS were scanned at 70 eV on a Jeol D-300 instrument. Column chromatography was performed on silica gel (Merck, 60-120 mesh) and thin-layer chromatography on silica gel G coated TLC plates (Merck). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagent.

3.2 Plant Material

The tubers of *Cyperus rotundus* L. were collected from West Champaran, Bihar, (India) and identified by Dr. H. B. Singh, Scientist F and Head, Raw Materials, Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen (No. NISCAIR/RHMD/Consult/-2008-09/1114/145) was deposited in The Herbarium of NISCAIR, New Delhi.

3.3 Extraction and isolation

Tubers of *Cyperus rotundus* (1 kg) were shade dried, coarsely powdered and extracted exhaustively with methanol. The methanolic extract was concentrated under reduced pressure in a Buchi rotavapor to obtain a dark green viscous mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous mass was dissolved in little amount of methanol and adsorbed on silica gel (60-120 mesh) for column for preparation of slurry. The slurry (85.5 g) was air-dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3), pure chloroform and finally the mixture of chloroform and methanol (99:1, 98:2, 96:4, 95:5, 97:3, 9:1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The isolated compounds were recrystallized to get the following compounds:

3.3.1 n- Triacont-1-ol-21-one 1 (1)

Elution of the column with chloroform- methanol mixture (99:1) afforded colourless mass of **1,** recrystallized from acetone, 820 mg (0.96 % yield). R_f: 0.42 ($CHCl_3$-MeOH, 5:3. m.p: 144-146 °C. IR ν_{max} (KBr): 3437, 2927, 2866, 1703, 1645, 1458, 1376, 1243, 1028, 889, 755 cm^{-1}. ^{1}HNMR ($CDCl_3$): δ 3.30 (2H, brs, H_2-2), 2.14 (2H, m, H_2-20), 2.01 (2H, m, H_2-22), 1.63 (2H, m, CH_2), 1.59 (2 H, m, CH_2), 1.46 (2H, m, CH_2), 1.20 (36 H, brs, 18 × CH_2), 1.09 (4H, m, 2 × CH_2), 1.03 (4H, m, 2 × CH_2), 0.84 (3H, t, *J*= 6.5 Hz, Me-30); ^{13}C NMR ($CDCl_3$): δ 207.13 (C-21), 60.61 (C-1), 33.46 (CH_2), 28.61 (19 × CH_2), 25.17 (CH_2), 24.20 (CH_2), 14.36 (Me-30). +ve ion FAB MS *m/z* (*rel. int.*): 452 $[M]^+$ (C_{30} $H_{60}O_2$) (5.3), 325 (5.7), 297 (10.8), 155 (20.5), 127 (38.5).

3.3.2 18-Epi-α- amyrin glucuronoside (2)

Elution of the column with chloroform- methanol (97:3) yielded colourless crystal of **2**, recrystallized from methanol, 0.74g (0.87%). R_f: 0.54 ($CHCl_3$). m.p: 251-252 °C. UV λ_{max} (MeOH): 207 nm (log ε 4.9). IR ν_{max} (KBr): 3350, 2939, 2852, 1691, 1645, 1458, 1374, 1225, 1044, 997 cm^{-1}. ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6): see Table-1. +ve ion FAB MS *m/z* (*rel. int.*): 602 $[M]^+$ ($C_{36}H_{58}O_7$) (2.7), 425 (41.2), 390 (100), 219 (10.1), 207 (22.7), 203 (19.2), 192 (24.5), 189 (16.0), 188 (16.8), 177 (12.8), 174 (23.5), 173 (11.5), 162 (14.2), 159 (33.8), 158 (29.1), 147 (33.6), 144 (38.25), 132 (41.5), 129 (22.8), 95 (40.5).

Hydrolysis of **2**: Compound **2** (20 mg) was dissolved in MeOH and 2N HCl (1:1) and heated till half volume was left. The solution was dried under reduced pressure and the residue was dissolved in water. It was extracted with EtOAc (3 × 10 ml), the organic phase washed with H_2O (2 × 10 ml), dried over anhydrous Na_2SO_4 and evaporated to give 18-Epi-α- amyrin, MS *m/z* 426 $[M]^+$ ($C_{30}H_{50}O$) (5.2). The aqueous phase was concentrated and analyzed with paper chromatography along with standard samples of monosacchrides. *n*-Butanol: ethanol: water (4:1:2.2) was used as the developing solvent system. The paper was sprayed with aniline hydrogen phthalate. The sugar was identified as D-glucuronic acid (R_f. 0.33).

3.3.3 Oleanolic acid arabinoside (3)

Elution of the column with chloroform- methanol (19:1) yielded colourless crystal of **3**, recrystallized from methanol, 1.62 g (1.90%). R_f : 0.45 ($CHCl_3$:MeOH : 4:1). m.p: 239-240°C. UV λ_{max} (MeOH): 207 nm (log ε 5.3). IR ν_{max} (KBr): 3424, 2927, 2851, 2361, 1705, 1646, 1460, 1375 cm^{-1}. ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6): see Table- 2. +ve ion FAB MS *m/z* (*rel. int.*): 588 $[M]^+$ ($C_{35}H_{56}O_7$) (1.1), 455 $[M\text{-Ara}]^+$ ($C_{30}H_{48}O_3$) (61.8), 425 (9.8), 411 (11.2), 395 (11.3), 248 (100), 207 (36.8), 203 (99.8), 192 (45.6), 189 (72.9), 188 (31.5), 177 (25.1), 174 (32.7), 173 (30.6), 162 (34.1), 159 (38.6), 158 (28.1), 147 (48.1), 144 (42.3), 132 (68.3), 129 (28.7), 119 (83.7), 105 (72.4).

Hydrolysis of **3**: Compound **3** (20 mg) was dissolved in MeOH and 2N HCl (1:1) and heated till half volume was left. The solution was dried under reduced pressure and the residue was dissolved in EtOAc. The organic phase was washed with H_2O (2 × 10 ml), dried over anhydrous Na_2SO_4 and evaporated to give oleanolic acid, m.p. 309-310 °C. The aqueous phase was concentrated and analyzed with paper chromatography along with standard samples of monosacchrides. *n*-Butanol: ethanol: water (4:1:2.2) was used as the developing solvent system. The paper was sprayed with aniline hydrogen phthalate. The sugar was identified as D-arabinose (R_f. 0.22).

3.3.4 α - Amyrin glucopyranoside (4)

Elution of the column with chloroform-methanol (93:7), yielded colourless crystal of 1, recrystallized from methanol, 3.81 g (2.21%). R_f: 0.72 ($CHCl_3$: MeOH: 5:1). m.p: 229-230 °C. UV λ_{max} (MeOH): 208 nm (log ε 5.6). IR ν_{max} (KBr): 3440, 2925, 2362, 1645, 1550, 1469, 1375, 1220, 1048 cm^{-1}. ^1H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6): see Table-3. +ve ion FAB MS *m/z* (*rel. int.*): 588 $[M]^+$ ($C_{36}H_{60}O_6$) (2.6), 426 (5.2), 395 (10.2), 219 (32.5), 207 (11.5), 203 (24.5), 192 (23.6), 189 (13.1), 188 (7.5), 177 (19.8), 174 (28.8), 173 (30.1), 162 (35.3), 159 (53.2), 158 (33.6), 147 (46.2), 144 (33.7), 132 (35.2), 129 (85.2), 111 (62.0), 105 (89.3).

Hydrolysis of **4**: Compound **4** (15 mg) was dissolved in MeOH and 2N HCl (1:1) and heated till half of the volume was left. The solution was then dried under reduced pressure and the residue was dissolved in water and extracted with EtOAc (3 × 10 ml). The organic phase was washed with H_2O (2 × 10 ml), dried over anhydrous Na_2SO_4 and evaporated to give α–amyrin, m.p. 196-197 °C. The aqueous phase was concentrated and analyzed with paper chromatography along with standard samples of monosaccharides. *n*-butanol: ethanol: water (4:1:2.2) was used as the developing solvent system. The paper was sprayed with aniline hydrogen phthalate. The sugar was identified as D-glucose (R_f. 0.16)

3.3.5 β - Amyrin glucopyranoside (5)

Elution of the column with chloroform- methanol (91:9), yielded colourless crystal of 2, recrystallized from methanol, 1.96 g (1.08%). R_f: 0.61 ($CHCl_3$- MeOH: 4:1). m.p: 221-222 °C. UV λ_{max} (MeOH): 208 nm (log ε 5.5). IR ν_{max} (KBr): 3394, 2925, 2852, 1647, 1458, 1381, 1258, 1135, 1045, 921 cm^{-1}. ^1H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6): see Table-4. +ve ion FAB MS *m/z* (*rel. int.*): 588 $[M]^+$ ($C_{36}H_{60}O_6$) (11.5), 426 (5.6), 395 (12.8), 219 (9.1), 207 (2.7), 203 (9.6), 192 (4.5), 189 (1.8), 188 (3.4), 177 (2.8), 174 (13.5), 173 (13.9), 162 (4.2), 159 (29.3), 147 (23.6), 144 (29.2), 132 (31.5), 129 (22.8), 105 (76.3), 95 (100).

Hydrolysis of **5**: Compound **5** (20 mg) was dissolved in MeOH and 2N HCl (1:1) and heated till half of the volume was left. The solution was dried under reduced pressure and the residue was dissolved in water and extracted with EtOAc (3 × 10 ml). The organic phase was washed with H_2O (2 × 10 ml), dried over anhydrous Na_2SO_4 and evaporated to give β–amyrin, m.p. 193-195 °C. The aqueous phase was concentrated and analyzed with paper chromatography along with standard samples of monosaccharides. *n*-butanol: ethanol: water (4:1:2.2) was used as the developing solvent system. The paper was sprayed with aniline hydrogen phthalate. The sugar was identified as D-glucose (R_f. 0.16).

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