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STRUCTURE ELUCIDATION OF FOUR NEW TRITERPENOID OLIGOGLYCOSIDES FROM ANAGALLIS ARVENSIS

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Abstract - Besides characterisation of desglucoanagalloside, anagallisins A,B,D and E, four new triterpenoid oligoglycosides isolated from the aerial part of Anagallis arvensis were respectively defined to be anagalligenin B 3-O-{β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl (1→4)-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl (1→2)]-α-L-arabinopyranoside} (1), anagalligenone 3-O-{β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl (1→4)-[β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl (1→2)]-α-L-arabinopyranoside} (2), anagalligenone 3-O-{β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl (1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranoside} (4) and anagalligenin B-3-O-{β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→4)]-α-L-arabinopyranoside} (5). The structural features were elucidated by a combination of fast - atom - bombardment mass spectrometry, strategic chemical degradation, and ^{1}H and ^{13}C NMR spectroscopy.

Anagallis arvensis is a small herb which occurs wild in many parts of India particularly in Gangetic plain[1]. The plant has been reported to possess in vitro antiviral activity against Herpes simplex virus type I and polio virus[2-4]. The antiviral activity of the plant has been located in the saponin fraction. Isolation of a few triterpenoid saponins and their chemical characterization have been reported[5]. The wide occurrence of the herb coupled with the reported biological activities of the saponin fraction made the plant ideal target for isolation and structure elucidation of the saponins. This paper reports the isolation and characterization of five triterpenoid oligoglycosides of potential biological interest four of which turned out to be new glycosides.

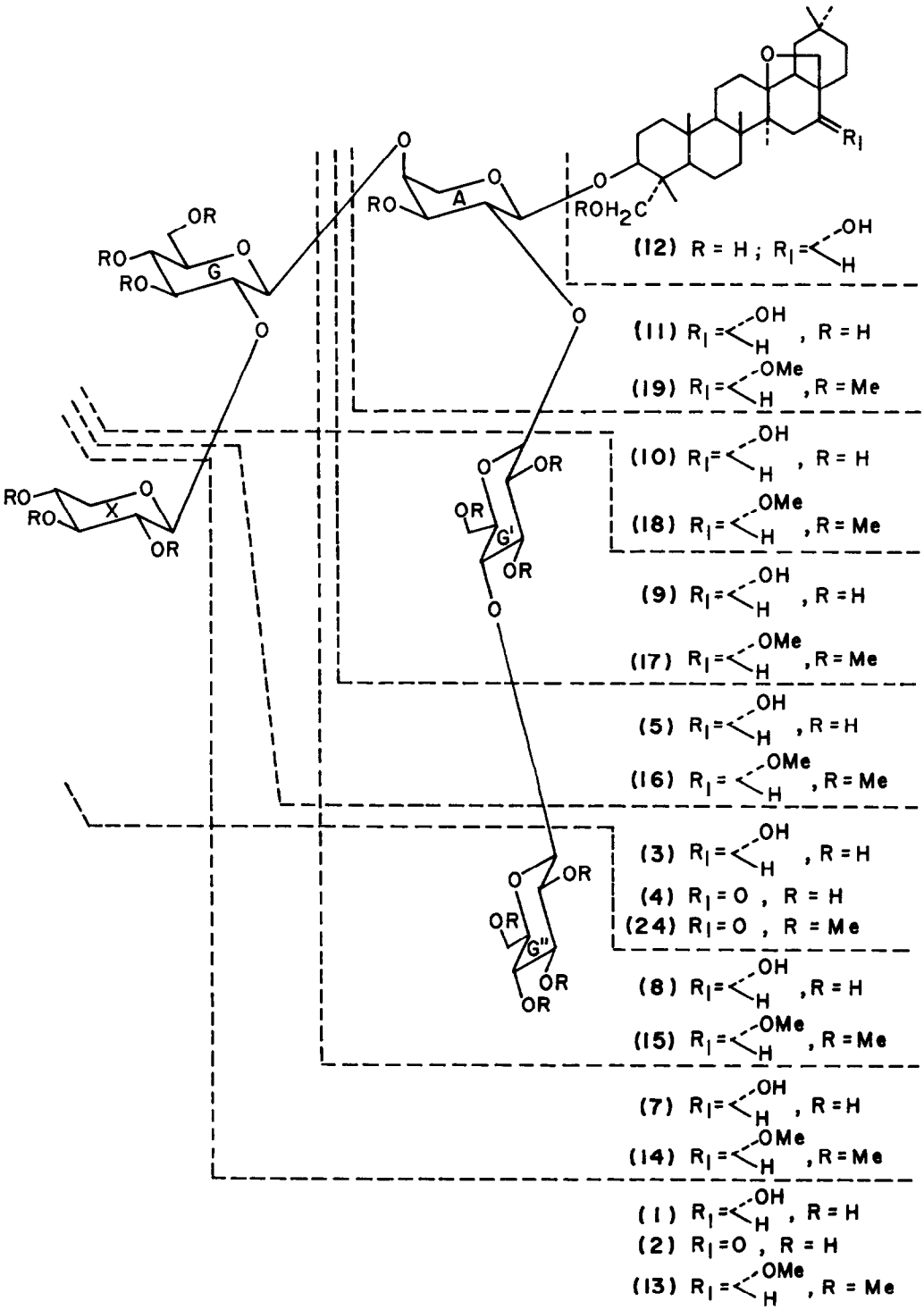
RESULTS AND DISCUSSION

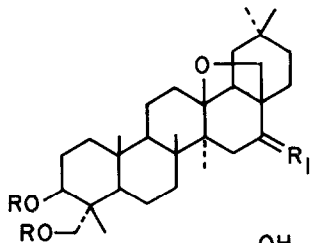
The $Bu^{n}OH$ soluble fraction of MeOH extract of the whole plant on repeated chromatographic purification on silica gel column followed by HPLC separation yielded five pure glycosides designated anagallisins A-E (1-5) according to decreasing order of their polarity. All the five saponins gave positive Liebermann-Burchard test for triterpenoids and

Molisch test for sugars. Anagallisin A (1) on acid hydrolysis liberated an aglycone identified as 23-hydroxyprimulagenin A (6) by comparison of its physical and spectral data with those of an authentic sample[4]. The monosaccharide constituents were identified as D-glucose, D-xylose and L-arabinose by descending paper chromatography and GLC by comparison with authentic samples. The absolute configuration of the L-arabinose unit was confirmed by its isolation and determination of its specific rotation. The aglycone, however, appeared to be an artefact formed during acid hydrolysis of the saponin as the ^{13}C NMR spectrum of the saponin did not show any signal assignable to an olefinic carbon. As such, attempts were made to isolate the genuine aglycone by application of the newer technique of hydrolysis using alcoholic-alkali metal solution[6]. Although the use of alcoholic-alkali metal solution having a trace of water[7] yielded the prosapogenins (5,7-11) in isolable yields, the aglycone (12) could not be isolated by this technique apparently due to its formation in traces. However, the use of Bu^nOH - Na metal at water-bath temperature (95°C) for 40 h afforded the aglycone (12) as the major product. The m.p., $[\alpha]_D$ values of compound (12) were found to be identical with those reported for anagalligenin B[5,8]. The ^1H and ^{13}C NMR data were compatible with its structure. Assignments of the ^{13}C values were made by the application of known chemical shift rules[9], comparison with the ^{13}C data of androsacenol[10], and the glycosylated anagalligenin B[5]. Fast-atom-bombardment mass spectrometry (FAB-MS)[11,12] was employed for the determination of the molecular weight and sequence of the monosaccharide units in the glycone portion of anagallisin A (1). Its positive FAB-MS displayed ion peaks at $\underline{m}/\underline{z}$ 1247, 1225 and 457 ascribed to $[M+Na]^+$, $[M+H]^+$ and $[M+H$ - sugar moiety - $H_2O]^+$ respectively. The molecular weight of the saponin was further confirmed by determination of the positive FAB-MS by addition of KCl[13] which showed an intense ion at $\underline{m}/\underline{z}$ 1263 assigned to $[M+K]^+$. The negative FAB-MS exhibited various ion peaks which are shown in Table 1. Fragmentations corresponding to the losses of pentose, hexose, hexose-pentose, two hexoses-pentose, three hexoses-pentose and three hexoses - two pentoses from $[M-H]^-$ ion were observed which indicated that anagallisin A (1) contains a pentasaccharide moiety possessing the following monosaccharide sequences :

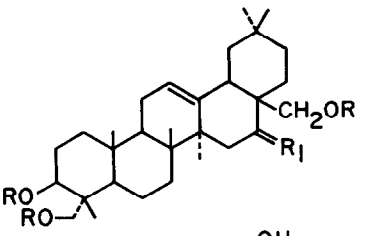
Pentose - hexose - pentose - S
|
hexose - hexose

S = anagalligenin B, hexose = glucose
and pentose = arabinose or xylose


(12) R = H ; R1 = OH
(11) R1 = OH , R = H
(19) R1 = OMe , R = Me
(10) R1 = OH , R = H
(18) R1 = OMe , R = Me
(9) R1 = OH , R = H
(17) R1 = OMe , R = Me
(5) R1 = OH , R = H
(16) R1 = OMe , R = Me
(3) R1 = OH , R = H
(4) R1 = O , R = H
(24) R1 = O , R = Me
(8) R1 = OH , R = H
(15) R1 = OMe , R = Me
(7) R1 = OH , R = H
(14) R1 = OMe , R = Me
(1) R1 = OH , R = H
(2) R1 = O , R = H
(13) R1 = OMe , R = Me



(12) R = H; R_1 = 
(20) R = H; R_1 = O
(22) R = Ac; R_1 = 
(23) R = Ac; R_1 = O



(6) R = H; R_1 = 
(21) R = H; R_1 = O
(25) R = Ac, R_1 = O

To determine which of the two pentoses are linked to the aglycone (**12**) the saponin was treated with sodium metaperiodate and worked up for detection of any monosaccharide present in the hydrolysate. L-arabinose could be detected as the only sugar constituent suggesting that the arabinose moiety of the saponin contains no vicinal diol and is linked to the aglycone. The intersugar linkages were revealed by permethylation, partial hydrolysis and identification of the partially methylated sugars. Thus permethylation of anagallisin A with NaH - MeI in hexamethylphosphoric triamide (HMPA) yielded the permethylate (**13**), which on acid hydrolysis yielded 2,3,4,6-tetra-O-methyl-D-glucose, 3,4,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-xylose and 3-O-methyl-L-arabinose. Moreover, partial hydrolysis of the saponin with alcoholic - alkali metal solution as mentioned above afforded six prosapogenins (**5,7-11**) which on permethylation yielded six permethylates (**14-19**). Acid hydrolysis of the permethylates yielded various partially methylated sugars (Table 2) identified by GLC of their alditol acetates using authentic samples.

β-Configuration ($^4C_1$ conformation) for the glucopyranosyl units and the xylopyranosyl, and α-configuration ($^1C_4$ conformation) for the arabinopyranoside were inferred from the J values of the respective anomeric protons in the ^1H NMR spectrum of the permethylate (**13**). The attachment of the carbohydrate moiety at the C-3 of the aglycone (**12**) was revealed from the ^{13}C NMR chemical shifts of anagallisin A and its aglycone (Table 3) taking into consideration the glycosylation shift values[14,15]. The carbon atoms C-2, C-3 and C-4 of saponin (**1**) were shifted by - 1.3, 6.5 and 0.7 p.p.m. respectively in comparison to the corresponding signals of the aglycone (**12**). The intersugar linkages were also indicated by the shifts of the α-carbon atoms as well as those of adjacent carbon atoms

Table 1. Characteristic mass spectral ions in the positive and negative FAB spectra of anagallisin A (**1**), anagallisin B (**2**), anagallisin C (**3**), anagallisin D (**4**) and anagallisin E (**5**).

Saponin	Significant ions (relative intensity) and assignment
1	m/z[a] 1247 (100) $[M+Na]^+$, 1225(37)$[M+H]^+$, 457(55)$[M+H-S-H_2O]^+$, 455(50), 439(75) and 421(30). m/z[b] 1223(100)$[M-H]^-$, 109(16.2)$[m-H-x]^-$, 1061(26.9)$[M-H-g]^-$, 929(8.7)$[M-H-g-x]^-$, 767(20)$[M-H-2g-x]^-$, 749(2.8), 747(3.4), 719(2.5), 627(5.0), 605(7.5)$[M-H-3g-x]^-$, 587(2.5), 585(3.7), 571(6.2), 569(4.4), 527(7.5), 473(5.6)$[M-H-3g-x-ar]^-$, 455(4.1), 453(7.2), 441(8.4), 439(5.6), 365(6.2), 249(12.4), 221(35.6) and 219 (19.4) m/z[c] 1263(100)$[m+K]^+$, 1131(18)$[M+K-x]^+$, 1083(16)$[M+K-g-H_2O]^+$, 1026(10)$[M+K-g-H_2O]^+$, 951$[M+K-x-g-H_2O]^+$, 880(5), 807(11) $[M+K-x-2g]^+$, 645(14)$[M+K-x-3g]^+$, 611(14), 457(12)$[M+K-S-H_2O]^+$,, 439(13)$[M+K-S-2H_2O]^+$, 407(22), 368(75), 311(28) and 191(86)
2	m/z[a] 1245(100)$[M+Na]^+$, 1223(87)$[M+H]^+$, 1113(25)$[M+Na-x]^+$, 1083(12)$[M+Na-g]^+$, 767(62)$[M+H-2g-x]^+$, 605(20)$[M+H-3g-x]^+$, 473(50)$[M+H-3g-x-ar]^+$ and 455(58)$[M+H-S-H_2O]^+$
3	m/z[a] 1085(10)$[M+Na]^+$, 1063(28)$[M+H]^+$, 769(10)$[M+H-x-g]^+$,589(17) $[M+H-genin]^+$, 457$[M+H-S-H_2O]^+$ and 439$[M+H-S-2H_2O]^+$ m/z[b] 1061(100)$[M-H]^-$, 929(34.2)$[M-H-x]^-$, 901(8), 767(22.4) $[M-H-x-g]^-$, 605(6)$[M-H-x-2g]^-$, 585(5.8)$[M-H-genin-2H]^-$, 567 (5.8), 473(4.2)$[M-H-x-2g-ar]^-$, 455(3.9)$[M-H-x-2g-ar-H_2O]^-$,453 (4.5), 441(7.1), 439(5.3), 407(6.6), 365(12.6), 249(14.5), 233(15), 221(15.3) and 219(15)
4	m/z[c] 1099(100)$[M+K]^+$, 1061(12)$[M+H]^+$, 967(8)$[M+K-x]^+$, 937(7)$[M+K-g]^+$, 805(6)$[M+K-x-g]^+$, 643(5)$[M+K-x-2g]^+$ and 455 (20)$[M+K-S-H_2O]^+$
5	m/z[b] 929(100)$[M-H]^-$, 767(18)$[M-H-g]^-$, 605(7)$[M-H-2g]^-$, 473 (5.4)$[M-H-S]^-$ and 455(15)$[M-H-S-H_2O]^-$

[a]positive FAB, [b]negative FAB, [c]KCl added to the sample in positive FAB, g = glucose, x = xylose, ar = arabinose, S = sugar moiety.

(β-shift) (Table 3), Moreover, identification of the various partially methylated alditol acetates obtained from the permethylates(**14-19**)(Table 2) disclosed the points of linkages. Thus the structure of anagallisin A is

Table 2 : Partially methylated sugars obtained by acid hydrolysis of the permethylated compound (**13-19,24**) and identified by GLC of their alditol acetates.

Compound	Partially methylated sugars	$(R_t)^{*17,18}$
13	2,3,4,6-tetra-O-methyl-D-glucose	1
	3,4,6-tri-O-methyl-D-glucose	1.90
	2,3,6-tri-O-methyl-D-glucose	2.30
	2,3,4-tri-O-methyl-D-xylose	0.54
	3-O-methyl-L-arabinose	1.42
14	2,3,4,6-tetra-O-methyl-D-glucose	1
	2,3,6-tri-O-methyl-D-glucose	2.34
	3-O-methyl-L-arabinose	1.45
15	2,3,4,6-tetra-O-methyl-D-glucose	1
	3,4-di-O-methyl-L-arabinose	1.36
	2,3,6-tri-O-methyl-D-glucose	2.30
16	2,3,4,6-tetra-O-methyl-D-glucose	1
	3-O-methyl-L-arabinose	1.45
17	2,3,4,6-tetra-O-methyl-D-glucose	1
	3,4-di-O-methyl-L-arabinose	1.35
18	2,3,4,6-tetra-O-methyl-D-glucose	1
	2,3-di-O-methyl-L-arabinose	1.03
19	2,3,4-tri-O-methyl-L-arabinose	0.55
24	2,3,4,6-tetra-O-methyl-D-glucose	1
	3,4,6-tri-O-methyl-D-glucose	1.86
	2,3,4-tri-O-methyl-D-xylose	0.55
	3-O-methyl-L-arabinose	1.47

*Retention times relative to 1,5, Di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol.

proposed to be anagallıgenin B 3-O-β-D-xylopyranosyl (1⟶2)-β-D-glucopyranosyl (1⟶4)-[β-D-glucopyranosyl (1⟶4)-β-D-glucopyranosyl (1⟶2)]-α-L-arabinopyranoside (**1**).

The FAB-MS of anagallisin B (**2**) displayed ion peaks at m/z 1245 and 1223 ascribed to $[M+Na]^+$ and $[M+H]^+$ respectively. These values and its other fragment ions when compared with those of anagallisin A (**1**) (Table

Table 3. Chemical shifts [δ_C (± 0.1)] of anagalligenin B (**12**), anagallisin A (**1**), anagallisin C (**3**), anagallisin E (**5**), anagalligenone (**20**), anagallisin B (**2**) and anagallisin D (**4**)

Carbon No.	(12)[a]	(1)[b]	(3)[b]	(5)[b]	(20)[a]	(2)[b]	(4)[b]
1	38.8	39.3	39.1	39.1	38.7	39.7	39.1
2	27.2	25.9	26.0	25.8	27.0	26.0	25.9
3	76.2	82.7	82.7	82.7	76.2	82.0	82.3
4	42.8	43.5	43.5	43.2	42.7	43.6	43.6
5	49.5	48.0	48.0	48.3	49.6	48.5	47.8
6	18.5	19.5	19.4	19.1	18.6	18.9	18.8
7	33.3[c]	33.1[c]	33.1[c]	33.2	33.4	33.5	33.7
8	42.0	42.5	42.5	42.5	42.0	42.9	43.0
9	50.1	50.7	50.7	50.6	50.0	50.5	50.4
10	36.8	36.9	36.9	36.7	36.6	36.6	36.7
11	18.5	19.5	19.6	19.4	17.8	17.8	17.5
12	32.8[c]	32.9[c]	32.9[c]	31.7	31.6	31.9	31.9
13	86.4	86.5	86.5	86.3	86.2	86.1	86.3
14	44.4	44.6	44.6	44.4	49.7	49.8	50.0
15	34.4	34.3	34.3	34.3	45.4	45.5	45.8
16	77.4	77.4	77.4	77.4	213.3	212.1	212.0
17	44.7	44.6	44.6	44.8	56.1	56.1	56.2
18	51.4	51.5	51.6	51.5	54.5	55.5	54.6
19	39.4	39.3	39.4	39.4	40.0	40.1	40.2
20	31.7	31.7	31.7	31.7	31.6	31.7	31.7
21	36.8	36.9	36.9	36.8	35.2	35.2	35.7
22	29.8	30.0	30.2	29.9	24.8	24.7	25.0
23	69.2	65.1	65.1	65.1	71.6	64.7	64.9
24	13.1	13.0	13.1	13.0	11.3	13.2	13.1
25	16.9	16.9	17.0	16.9	16.3	16.9	16.8
26	17.6	17.7	17.8	17.7	17.8	17.5	17.6
27	18.6	18.5	18.5	18.5	21.7	21.7	21.7
28	77.8	77.7	77.5	77.4	75.1	77.3	77.5
29	24.6	24.7	24.8	24.8	23.6	24.9	23.5
30	33.5	33.6	33.7	33.6	33.3	33.2	33.4
A-1		103.4	103.6	103.9		103.6	103.6
A-2		80.1	80.3	80.5		80.5	80.3
A-3		73.0	73.2	73.5		72.8	73.1
A-4		75.7[d]	75.5[d]	75.5[d]		75.9[d]	75.6[d]

Carbon No.	(12)[a]	(1)[b]	(3)[b]	(5)[b]	(20)[a]	(2)[b]	(4)[b]
A-5		64.0	64.3	64.2		64.0	64.2
G-1		104.2[e]	103.8	104.0[e]		104.8[f]	103.8[f]
G-2		83.3	84.6	74.5		84.0	84.7
G-3		77.9[f]	77.8[e]	78.0		78.1[e]	77.8[e]
G-4		71.4	71.4	71.5		71.4	71.2
G-5		78.0[f]	77.9[e]	78.1		78.2[e]	78.0
G-6		62.6	62.6	62.6		62.7	62.8
G'-1		104.5[e]	104.7	104.2[e]		104.9[f]	104.8[f]
G'-2		75.4[d]	75.8[d]	75.2[d]		76.0[d]	75.1[d]
G'-3		76.0	76.2	77.7		76.1[d]	77.8[e]
G'-4		78.1[f]	71.4	71.5		78.3[e]	71.5
G'-5		75.5[d]	77.9[e]	78.1		76.2[d]	78.0
G'-6		62.6	62.4	62.4		62.0	62.4
X-1		106.5	106.9			106.9	107.0
X-2		75.4	75.5[d]			74.9	75.9
X-3		77.9	77.8			77.3	77.4[e]
X-4		70.4	70.5			70.6	70.5
X-5		67.1	67.2			67.3	67.2
G"-1		104.5[e]				104.7[f]	
G"-2		74.5				74.8	
G"-3		76.9				77.3[e]	
G"-4		71.4				71.5	
G"-5		77.9[f]				78.2[e]	
G"=6		62.6				62.5	

[a]in $CDCl_3$, [b]in C_5D_5N, A = arabinose, X = xylose, G,G',G" = glucose c,d,e f may be reversed on each vertical column.

1) revealed that the aglycone (**20**) of saponin (**2**) possesses molecular weight of 472 i.e. two mass units less than that of aglycone (**12**). However, the composition of the carbohydrate constituents and their linkages in the glycone part of both the saponins appeared to be the same by the FAB-MS (Table 1) and ^{13}C NMR data (Table 3). Although acid hydrolysis of anagallisin B (**2**) yielded the rearranged aglycone (**21**) which was characterised from its MS and ^1H NMR of its acetate (**25**), treatment of saponin (**2**) in Bu^nOH with Na metal at 95°C for 40 h furnished the genuine aglycone, anagaligenone (**20**). Its characterization was accomplished by its MS, ^1H and ^{13}C NMR data. The molecular ion of compound (**20**) was displayed at m/z 472. The other fragment ions (see Experimental) and the

^{13}C NMR data which were assigned by comparison with those of anagalligenin B are compatible with the structure (**20**). That anagalligenone (**20**) is a genuine sapogenol and not an artifact was suggested by the ^{13}C NMR spectrum of saponin (**2**) which showed besides the carbonyl signal at 212.1 attributed to C-16, other signals comparable to those of compound (**20**). It may be mentioned that this is the first report of isolation of anagalligenone from a natural source. On the basis of the foregoing evidence the structure of anagallisin B has been defined as anagalligenone 3-O--β-D-xylopyranosyl (1⟶2)-β-D-glucopyranosyl (1⟶4)-[β-D-glucopyranosyl (1⟶4)-β-D-glucopyranosyl (1⟶2)]-α-L-arabinopyranoside (**2**).

Anagallisin C (**3**) showed in its FAB-MS the $[M+Na]^+$ and $[M+H]^+$ ions at m/z 1085 and 1063 respectively. Moreover, on acid hydrolysis it afforded D-glucose, D-xylose and L-arabinose as carbohydrate constituents and 23-hydroxyprimulagenin A (**6**) as the aglycone. These evidences as well as the ^{13}C NMR chemical shifts of compound (**3**) suggested its identity with desglucoanagalloside B[5].

Anagallisin D (**4**) showed in its FAB-MS ion peaks at m/z 1099 and 1061 ascribed to $[M+K]^+$ and $[M+H]^+$ respectively. Hydrolysis and permethylation studies in conjunction with the ^{13}C NMR data led to the establishment of its structure as anagalligenone 3-O-{β-D-xylopyranosyl (1⟶2)-β-D-glucopyranosyl (1⟶4)-[β-D-glucopyranosyl (1⟶2)]-α-L-arabinopyranoside}(**4**).

Anagallisin E (**5**) turned out to be a trisaccharide of anagalligenin B (**12**) containing L-arabinose and D-glucose. The structure of the trisaccharide moiety was evident from permethylation studies as well as ^{13}C NMR chemical shifts of the saponin. Finally the structure of the saponin was confirmed by its direct comparison with the prosapogenin (**5**) obtained by partial hydrolysis of anagallisin A as described previously. Consequently, anagallisin E is defined as anagalligenin B 3-O-β-D-glucopyranosyl (1⟶2)-[β-D-glucopyranosyl (1⟶4)]-α-L-arabinopyranoside (**5**).

EXPERIMENTAL

The plant material was identified at Indian Botanic Garden, Howrah and a voucher specimen has been deposited at the herbarium of IICB.

All melting points were measured on a capillary melting point apparatus and are uncorrected. TLC was carried out on silica gel G with the solvent $CHCl_3$-MeOH-H_2O (60:30:5). Paper chromatography was done on Whatman paper No.1 with solvent system Bu^nOH-C_5H_5N-H_2O (6:4:3); a saturated solution of aniline oxalate in water was used for staining. GLC was performed on a Hewlett - Packard model 5730A instrument using the columns(i) ECNSS-M, 3% on Gas Chrome Q at 190°C for alditol acetates

and (ii) OV-225 on Gas Chrome Q at 195°C for partially methylated alditol acetates. Optical rotations were measured on a JASCO DPI - 360 digital polarimeter. High performance liquid chromatography (HPLC) was performed on a spectra- physics model SP 8000B instrument with a column of Spherisorb S-10-ODS and a Micromeritics 771 refractive index detector in MeOH-H_2O (75:25) as mobile phase. ^1H NMR spectra were recorded on a JEOL FX -100 (99.6 MHz) instrument in $CDCl_3$. ^{13}C NMR spectra were recorded either on a JEOL FX-100 spectrometer operating at 25.05 MHz or on a Bruker spectrometer (300 MHz) operating at 75 MHz in C_5D_5N and $CDCl_3$ respectively with tetramethylsilane as internal standard. Positive Fast-atom-bombardment mass spectra (FAB-MS) were obtained on a VG-ZAB-SE mass spectrometer equipped with a FAB source operating at an accelerating voltage of 8 kV. Samples were dissolved in [$^2H_6$] DMSO (2-10 μg μl^{-1}) and deposited on a FAB probe trip. A thin layer of either glycerol or thioglycerol was applied to the probe tip containing the samples and mixed thoroughly before insertioon into the source. The primary atom (xenon) was produced using a saddle-field ion source operating at a tube current of 1-1.5 mA at an energy of 8 kV. For the salt addition technique, samples were dissolved in DMSO-d_6 (2-5 μg μl^{-1}) to which KCl was added such that the sample to salt ratio was about 1:3. A thin layer of a glycerol-thioglycerol mixture (50:50) was applied to the copper probe tip to which the sample solution containing salt was added and thoroughly mixed. The probe was then introduced into the sourcce of the mass spectrometer for data acquisition. Negative FAB-MS were obtained on a Kratos MS-9/50 TC spectrometer. The samples loaded on to the copper probe tip with glycerol, were bombarded with a fast atom beam of xenon produced by an Jon-Tech 11 NP atom gun operating at a potential of 9 kV. The spectra were recorded using a UV galvanometer recorder. Electron-impact mass spectra were recorded at 70 eV.

Isolation of the Saponins - The air dried powdered aerial part of A. arvensis (2 Kg) was successively extracted with petroleum ether (60-80°C), chloroform and methanol. The methanolic extract on removal of the solvent under reduced pressure was partitioned between water-Bu^nOH. The Bu^nOH extract was washed with water and evaporated to dryness under reduced pressure to give a dark brown mass (42 g). This was chromatographed on a column of silica gel (900 g), elution being performed with chloroform and various ratios of chloroform - methanol. A total of 122 fractions (each 250 ml) were collected and fractions giving similar spots on TLC were combined. These fractions were subjected to HPLC separation on a reversed phase Spherisorb S-10-ODS column with mo-

bile phase MeOH - H_2O (75:25). Thus anagallisin A (150 mg), anagallisin B (40 mg), anagallisin C (340 mg), anagallisin D (250 mg) and anagallisin E (25 mg) were obtained.

anagallisin A (1) - It was crystallized from MeOH to give microneedles, mp 244-246°C, $[\alpha]_D$ - 5.81° (c 0.5 in MeOH) (Found : C, 56.1; H, 7.9; $C_{53}H_{96}O_{27}\cdot H_2O$ requires C, 56.02; H, 7.94%).

Hydrolysis of Anagallisin A (1) - Compound (1) (150 mg) was hydrolysed with 2M-HCl in aq. MeOH (46 ml) on a water bath for 5 h. Usual work up followed by chromatographic purification on a silica gel column gave 23-hydrooxyprimulagenin A (6) which crystallized from MeOH as needles (45 mg), mp 240-244°C, $[\alpha]^D$ + 40.8° (c 0.3 in MeOH) (lit.[16] mp 246-255°C $[\alpha]_D$ + 47.6°). Its ^1H NMR and MS data were comparable to those of an authentic sample.

The filtrate from the hydrolysate was neutralised with Ag_2CO_3, filtered, and a portion of the filtrate was concentrated under reduced pressure and tested for carbohydrates by paper chromatography using authentic samples. Three spots corresponding to D-glucose, D-xylose and L-arabinose were obtained. That the arabinose was the L-enantiomer was confirmed by its actual isolation by preparative paper chromatography and comparison of specific rotation with that of authentic L-arabinose. The other portion of the concentrated filtrate was reduced with $NaBH_4$ and worked up as usual. The residue was acetylated with Ac_2O-pyridine (1:1), worked up in the usual manner and subjected to GLC analysis on column (1). Three peaks corresponding to glucitol acetate, xylitol acetate and arabinitol acetate were detected using authentic specimens.

Hydrolysis of Anagallisin A (1) with Bu^nOH - Na Metal - Compound (1) (200 mg) dissolved in spectroscopic grade Bu^nOH (18 ml) was treated with a solution of sodium metal (1.5 g) dissolved in spectroscopic grade Bu^nOH (20 ml) at 95°C for 40 h. Water was then added and the Bu^nOH layer separated, washed free from alkali, dried and subjected to chromatographic purification. The major product thus obtained was crystallized from MeOH to yield needles of anagalligenin B (**12**), mp 248-250°C, $[\alpha]_D$ + 7.4° (c 0.2 in MeOH) MS, m/z 474 (39%), 456 (M^+-H_2O) (10), 455 (M^+-H_2O-H) (27),425 ($M^+-H_2O-CH_2OH$) (13), 424 ($M^+-H_2O-CH_3OH$) (16), 407($M^+-2H_2O-CH_2OH$) (12), 250 (a) (43), 232 (a - H_2O) (70), 219 (a-CH_2OH) (100) and 201 (a-CH_2OH-H_2O) (65). The acetate (**22**) of compound (**12**) prepared by treatment with Ac_2O-pyridine in the usual way was obtained as an amorphous powder, ^1H NMR ($CDCl_3$)δ0.84 (3H,s) 0.96 (3H,s), 0.98 (3H,s), 1.00 (3H,s), 1.12 (3H, s), 1.16 (3H,s) (6xMe) 2.04 (3H,s), 2.08 (3H,s), 2.10 (3H,s) (3 x OAc)

3.24 (1H, d, J 8Hz, 28-H), 3.64 (1H, d, J 8Hz, 28-H), 3.70 and 3.90 (2H, ABq, J 12Hz, $-CH_2OAc$), 4.76 and 4.86 (d each, J 5, 10Hz, 3 - axial H) and 5.08 (1H, d-like, J 5Hz).

Periodate Oxidation of Anagallisin A (1) and Hydrolysis of the Product To a solution of compound (**1**) (30 mg) in 90% EtOH (4 ml) was added dropwise a solution of sodium metaperiodate (35 mg) in water (3 ml) and the mixture was stirred at 15°C for 3 h, kept at room temperature overnight and worked up in the usual way. The residue was hydrolysed with 2M-HCl and the aqueous phase was examined for carbohydrates by paper chromatography and GLC. Only one peak was obtained corresponding to L-arabinose.

Permethylation of Anagallisin A (1) and Hydrolysis of the Product - To a solution of compound (**1**) (50 mg) in HMPA (6 ml) was added NaH (270 mg) and MeI (7 ml) and stirred at room temperature for 3 h. The reaction mixture was extracted with diethyl ether. Usual work up and chromatographic purification on silica gel furnished the permethylate (**13**) (24 mg) as powder; ^{1}H NMR ($CDCl_3$) δ 4.06 (1H, d, J 5.5Hz, 1-H of arabinose unit), 4.30 (1H, d, J 7Hz, 1-H of glucose unit), 4.44 (1H, d, J 7Hz, 1-H of glucose unit), 4.56 (1H, d, J 7Hz, 1-H of glucose unit), 4.56 (1H, d, J 7Hz , 1-H of glucose unit), 4.65 (1H, d, J 6Hz, 1-H of xylose unit).

The permethylated product (**13**) (12 mg) was hydrolysed on being heated under reflux with 2M-HCl in aq. MeOH (6 ml) for 5 h. The reaction mixture was worked up as usual and the aqueous filtrate was neutralsed with Ag_2CO_3 and filtered. The filtrate was concentrated and then reduced with $NaBH_4$ to yield after usual work up a residue which was acetylated with Ac_2O-pyridine (1:1) at 90°C for 1 h and subjected to GLC analysis on column (ii). Five peaks were detected and identified to be those of alditol acetates of the methylated sugars as shown in Table 2.

Partial hydrolysis of Anagallisin A (1) with Alcoholic Alkali metal solution and methylation of the Prosapogenin mixture followed by their separation - Compound (**1**) (95 mg) in absolute ethanol (10 ml) was treated with sodium metal (1 g) in absolute ethanol (10 ml). To this solution two drops of water was added and left for 48 h. TLC examination of the mixture revealed 8 spots indicating formation of six prosapogenins. The mixture containing six prosapogenins, the aglycone (**12**) as well as compound (**1**) was methylated in the same manner as for (**1**) in the preceeding experiment to give a resinous substance (108 mg), which was subjected to preparative TLC on silica gel plates using developing solvent benzene-acetone (3:1) to give permethylated prosapogenins (**5**)(4.5

mg), (7) (6.4 mg), (8) (7.2 mg), (9) (3.6 mg), (10) (5.2 mg) and (11) (6.0 mg).

Permethylate (5) - ^{1}H NMR ($CDCl_3$) δ 4.18 (1H, d, J 5Hz, 1-H of arabinose unit), 4.36 (1H, d, J 7Hz, 1-H of glucose unit), 4.41 (1H, d, J 7Hz, 1-H of glucose unit). On acid hydrolysis it yielded the partially methylated sugars as shown in Table-2 identified by GLC analysis of the alditol acetates.

Permethylate (7) - ^{1}H NMR ($CDCl_3$) δ 4.20 (1H, d, J 5Hz, 1-H of arabinose unit), 4.38 (1H, d, J 6.5Hz, 1-H of glucose unit), 4.41 (1H, d, J 7Hz, 1-H of glucose unit), 4.43 (1H, d, J 7Hz, 1-H of glucose unit). This on hydrolysis liberated the methylated sugars shown in Table 2.

Permethylate (8) - ^{1}H NMR ($CDCl_3$) δ 4.19 (1H, d, J 5.5Hz, 1-H of arabinose unit), 4.35 (1H, d, J 7Hz, 1-H of glucose unit), 4.40 (1H, d, J 7Hz, 1-H of glucose unit). This permethylate on acid hydrolysis yielded methylated sugars as shown in Table 2.

Permethylate (9) - ^{1}H NMR ($CDCl_3$) δ 4.21 (1H, d, J 5Hz, 1-H of arabinose unit), 4.40 (1H, d, J 6.5Hz, 1-H of glucose unit). Acid hydrolysis of this permethylate furnished methylated sugars as shown in Table 2.

Permethylate (10) - ^{1}H NMR ($CDCl_3$) δ 4.19 (1H, d, J 5.5Hz, 1-H of arabinose unit), 4.41 (1H, d, J 7Hz, 1-H of glucose unit). On acid hydrolysis it furnished the methylated sugars as shown in Table 2.

Permethylate (11) - ^{1}H NMR ($CDCl_3$) δ 4.22 (1H, d, J 5Hz, 1-H of arabinose unit). The compound (11) on acid hydrolysis yielded the partially methylated arabinose shown in Table 2.

Anagallisin B (2) - The compound (2) was crystallized from MeOH to yield microneedles, mp 256-260°C, $[\alpha]_D$- 18.2° (c 0.5 in MeOH) (Found : C, 55.39; H, 7.90; $C_{58}H_{94}O_{27}.2H_2O$ requires C, 55.31; H, 7.84%).

Hydrolysis of Anagallisin B (2) - Compound (2) (90 mg) was hydrolyused with 2M-HCl in aq. MeOH (25 ml) under reflux for 4 h. The usual work-up followed by chromatographic purification yielded the ketone (21) which crystallized from acetone to give needles, mp 218-220°C, $[\alpha]_D$+ 19.9° (c 0.3 in $CHCl_3$); IR 3420-3300 (hydroxyl), 1705 cm^{-1} (ketone); ^{1}H NMR ($CDCl_3$)δ0.88 (6H,s), 0.90 (3H,s), 1.00 (3H,s), 1.04 (3H,s), 1.22 (3H,s) (togethr 6 x Me), 3.44, 3.62 (2H, each d, J 12Hz, 28-H_2), 3.70, 3.98 (2H, each d, J 12 Hz, 23-H_2), 5.44 (1H, t-like, 12-H); MS, m/z 472 (M^+), 454 (M^+- H_2O), 441 (M^+-CH_2OH) (Found : C, 76.20; H, 10.28; $C_{30}H_{48}O_5$ requires C, 76.22; H, 10.24%).

compound (**21**) (20 mg) was acetylated with Ac_2O (2 ml) and pyridine (1 ml) at water bath temperature for 1 h. Usual work up afforded the acetate (**25**), IR 1735, 1240 (OAc), 1705 cm^{-1} (CO); MS m/z 598 (M^+), 538 (M^+-AcOH), 525 (M^+-CH_2OAc), 465 (M^+-CH_2OAc - AcOH); ^1H NMR ($CDCl_3$) δ 0.84 (3H,s), 0.86(6H,s), 1.06 (3H,s), 1.20 (3H,s), 1.24 (3H,s) (together 6 x Me), 2.0 (3H,s), 2.02 (3H,s), 2.04 (3H,s) (together 3 x OAc), 3.72, 3.85 (2H, each d, J 12Hz, 28-H_2), 3.94, 4.49 (2H, each d, J 12Hz, 23-H_2), 4.73 , 4.84 (1H, each d, J 5, 10Hz), 5.44 (1H, t-like, 12-H) (Found : C, 72.24; H, 9.05; $C_{36}H_{54}O_7$ requires C, 72.21, H, 9.09%).

Hydrolysis of anagallisin B (**2**) (120 mg) with Bu^nOH (10 ml) and Na metal (1 g) as desscribed for anagallisin A (**1**) furnished anagalligenone (**20**) which crystallised from acetone in microneedles, mp 264-266°C, $[\alpha]_D$ - 9.16° (c 0.24 in $CHCl_3$); MS, m/z 472 (M^+, 12%), 454 (M^+- H_2O, 8), 442 (M^+- CH_2O, 44), 436 (M^+- $2H_2O$, 25), 248 (ion a, 18), 217 (a - CH_2OH, 100) and 223 (b, 55).

Acetylation of compound (**20**) with Ac_2O-pyridine in the usual way furnished the acetate (**23**) as an amorphous solid, $[\alpha]_D$ - 6.6° (c 0.6 in $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.84 (3H,s), 0.88 (3H,s), 0.94 (3H,s), 1.04 (3H,s), 1.24 (3H,s), 1.26 (3H,s) (together 6 x Me), 2.04, 2.08 (each s, 2 x OAc), 3.46 (1H, d, J 8Hz, 28-H), 3.80 (1H, d, J 8Hz, 28-H), 3.74, 3.90 (2H, ABq, J 12Hz, CH_2OAc), 4.72, 4.84 (d each, J 5, 9Hz, 3 - axial H).

Anagallisin C (3) - The compound (**13**) was crystallised from MeOH to yield microneedles mp 236-238°C, $[\alpha]_D$ - 3.2° (c 0.5 MeOH). The mp and $[\alpha]_D$ were not available from literature. However, the spectroscopic data (FAB-MS and ^{13}C NMR) were found to be in good agreement with those reported for desglucoanagalloside B[5].

Anagallisin D (4)) - The compound (**4**) crystallized from MeOH as microprisms, mp 256-260°C, $[\alpha]_D$ - 6.9° (c 0.4 in MeOH) (Found : C, 58.79; H, 8.0; $C_{52}H_{84}O_{22}$ requires C, 58.85; H, 7.98%). Hydrolysis of anagallisin D (**4**) (80 mg) with 2M-HCl (15 ml) aq. MeOH yielded olean -12-ene-3, 23, 28-trihydroxy-16-one (**21**) and sugar constituents indentified as D-glucose, D-xylose and L-arabinose by PC and GLC. Hydrolysis of anagallisin D (**4**) (60 mg) in Bu^nOH (12 ml) on treatment with a solution of Na-metal (1 g) with Bu^nOH (10 ml) at 95°C for 36 h furnished anagalligenone (**20**) (8 mg).

Permethylation of compound (**4**) (50 mg) using HMPA (7 ml), NaH (320 mg) and MeI (7 ml) in the usual way yielded a permethylate (**24**) as a powder (22 mg) ^1H NMR ($CDCl_3$) δ 4.32 (1H, d, J 5Hz, 1-H of arabinose

unit), 4.37 (1H, d, J 6.5Hz, 1-H of glucose unit), 4.42 (1H, d, J 7Hz, 1-H of glucose unit) and 4.70 (1H, d, J 6Hz, 1-H of xylose unit).

Hydrolysis of the permethylate (**24**) (15 mg) with 2M-HCl in aq. MeOH (5 ml) on water bath for 3 h yielded after usual work up alditol acetates of the methylated sugars shown in Table 2.

Anagallisin E (5) - The compound (**5**) crystallised from MeOH as microneedles (90 mg) mp 224-226°C, $[\alpha]_D$ - 6.8° (c 0.4 in MeOH) (Found : C, 60.6; H, 8.4; $C_{47}H_{78}O_{18}$ requires C, 60.62; H, 8.44%).

Hydrolysis of anagallisin E (**5**) (65 mg) with 2M-HCl in aq. MeOH (18 ml) under reflux for 4 h yielded 23-hydroxyprimulagenin A (12 mg) and D-glucose and L-arabinose as the sugar constituents.

Compound (**5**) (55 mg) was Permethylated as described previously to yield the permethylate (**16**) (60 mg) as white powder, ^1H NMR ($CDCl_3$) δ 4.22 (1H, d, J 5Hz, 1-H of arabinose), 4.24 (1H, d, J 7Hz, 1-H of glucose unit), 4.38 (1H, d, J 6.5Hz, 1-H of glucose unit).

Hydrolysis of the permethylkate (**16**) (35 mg) as described earlier furnished the methylated sugars as shown in Table 2.

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