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PENTACYCLIC TRITERPENOID SAPOGENOLS AND THEIR GLYCOSIDES FROM TERMINALIA BELLERICA

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Key words : Terminalia bellerica; combretaceae; bellericagenin A; bellericagenin B; bellericaside A; bellericaside B; triterpenoids; triterpenoid glycosides.

Abstract - Two new pentacyclic triterpene acids, bellericagenin A and bellericagenin B and their glycosides, bellericaside A and bellericaside B were isolated from the stem-bark of Terminalia bellerica and were respectively defined as 2α, 3β, 7α, 23-tetrahydroxyolean-12-en-28-oic acid, 2α, 3β, 19α, 23,24-pentahydroxyolean-12-en-28-oic acid, β-D-glucopyranosyl 2α, 3β, 7α, 23-tetrahydroxyolean-12-en-28-oate and β-D-galactopyranosyl 2α, 3β, 19α, 23, 24-pentahydroxyolean-12-en-28-oate based on their spectroscopic properties and some chemical transformations.

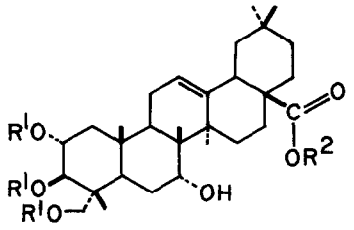
Terminalia bellerica Roxb is a reputed medicinal plant and occurs widely in the moist valleys of india[1]. In a previous communication[2] the isolation and structure elucidation of a new triterpene acid, belleric acid and its glucoside, bellericoside besides the identification of arjungenin and its glucoside, arjunglucoside I were reported. Biological screening of these compounds revealed their antibacterial property[3] which prompted us to investigate further for other similar constituents of the plant. We have been successful in isolating two further new triterpenoid sapogenols and their glycosides. This paper reports the isolation and structure elucidation of these biologically important natural products.

RESULTS AND DISCUSSION

The Bu^nOH soluble fraction of methanol extract of the defatted stem-bark of T. bellerica was separated into acidic and neutral fractions by treatment with a saturated solution of $NaHCO_3$. The acidic fraction on repeated chromatographic purification over silica gel column followed by preparative TLC separation afforded, besides arjungenin[2,4] and belleric acid[2], two new triterpenoid acids (Liebermann-Burchard and bicarbonate

tests) designated bellericagenins A(1) and B(2) according to increasing order of their polarity. The less polar acid, bellericagenin A(1), $C_{30}H_{48}O_6$ (elemental analysis, MS) on treatment with an ethereal solution of CH_2N_2 yielded a methyl ester (3) (δ3.61, 3H, s, $CO_2\underline{CH}_3$). The ester (3) on acetylation, furnished a methyl ester acetate (4) which showed in its ^1H NMR spectrum three acetoxy methyl signals at δ1.99, 2.03 and 2.07, strongly indicating the presence of three unhindered hydroxyl groups in bellericagenin A(1). Thus the nature of five out of six oxygen atoms in bellericagenin A was determined. That the remaining oxygen function is also hydroxyl in nature was ascertained from the ^{13}C NMR spectrum of bellericagenin A(1) which showed the presence of three secondary hydroxyls, one primary hydroxyl, an acid carbonyl and a trisubstituted double bond. As the spectrum exhibited six quaternary carbons below 60 ppm, its skeleton transpired to be that of oleanane. The spectrum further disclosed the presence of eight methylenes, three methines and six methyl carbons. The $\underline{M}_r$ of the methyl ester triacetate (4), determined by mass spectrometry was indicative of the presence of a free hydroxyl group. The MS of the compound (4) showed retro-Diels-Alder fragments typical of Δ^{12}-oleanene or ursene triterpenes[5]. Thus a peak at $\underline{m}/\underline{z}$ 262 was attributed to the r.D.A. fragment ion($\underline{b}$), involving rings D/E containing a carbomethoxy group. Consequently, all the four hydroxyl groups of bellericagenin A(1) were suggested to be present in the part containing rings A/B. The formation of other significant fragment ions could also be rationalized.

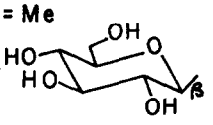
The presence of an α-glycol system in bellericagenin A(1) was indicated by the consumption of one mole of periodate per mole of the compound. This α-glycol system could be defined to be 2α, 3β-$(OH)_2$ by the ^1H NMR spectrum of the methyl ester triacetate (4) which exhibited signals at δ5.01(1H, d, $\underline{J}$ 11Hz) and 5.23(1H, td, $\underline{J}$, 6Hz) attributable to H-3α and H-2β respectively. The chemical shift and splitting (δ3.74, 1H, $\underline{J}$ 12 Hz and 3.95, 1H, d, $\underline{J}$ 12 Hz) of the CH_2OAc system disclosed that the primary hydroxyl group in compound (1) is located at C-23, as the CH_2OAc systems at C-24 and C-25 usually resonate at lower field[6-8] (~δ4.2 and 4.3 respectively). The possibility of the presence of a 26-CH_2OH group is very uncommon and therefore unlikely. The position of the fourth hydroxyl group in bellericagenin A(1) was ascertained as follows. The hindered nature of this hydroxyl group was evident by the formation of the triacetate (4) from the ester (3) by treatment with Ac_2O in pyridine at ambient temperature. As such considering the mass spectral informations as stated earlier, the presence of this hydroxyl group in the 6β, 7α or 11β position was indicated. However, the unchanged chemical shifts of C-12 and C-13 olefinic carbons ruled out the possibility of the occurrence of this hydroxyl group in the 11β position. As the physical and spectral data of compound (1) and its derivatives (3 and 4) differed noticeably from those of terminolic acid[9], the 6β-OH isomer and its corresponding deriatives, the presence of the hindered hydroxyl group at 7α position

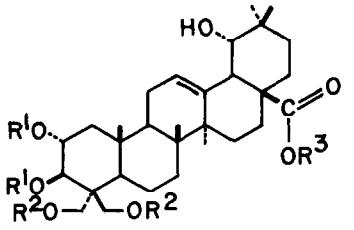


(1) $R^1 = R^2 = H$

(3) $R^1 = H, R^2 = Me$

(4) $R^1 = Ac, R^2 = Me$

(11) $R^1 = H, R^2 =$ 



(2) $R^1 = R^2 = R^3 = H$

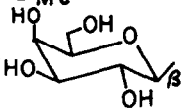
(5) $R^1 = R^2 = H, R^3 = Me$

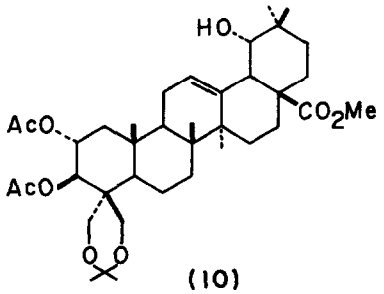
(6) $R^1 = R^2 = Ac, R^3 = Me$

(7) $R^1 = H, R^2 = CPh_3, R^3 = Me$

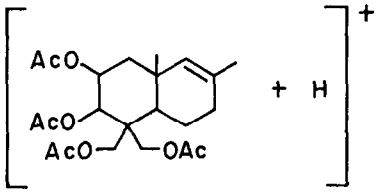
(8) $R^1 = Ac, R^2 = CPh_3, R^3 = Me$

(9) $R^1 = Ac, R^2 = H, R^3 = Me$

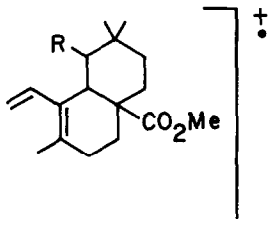
(12) $R^1 = R^2 = H, R^3 =$ 



(10)



$[a + H]^+$ m/z 425



(b) R = H; m/z 262

(b') R = OH; m/z 278

was indicated. Moreover, bellericagenin A (1) was found to be different from terminolic acid by direct comparison with an authentic sample. The presence of the 7α-hydroxyl group was also shown by the downfield shift of about 0.30 ppm for the C-27 methyl singlet in the ^{1}H NMR spectrum of compound (4) compared to an oleanene triterpene not containing a 7α-hydroxyl group. This shift can be explained by van der Waals deshielding effect[10] of the methyl group by the hydroxyl group as they are in a 1:3 diaxial relationship[7,11]. The location of the carboxyl group at C-17 was established by comparison of the ^{13}C-values of ring-D and E carbons of compound (1) with those of analogous triterpenoids[2,12]. The ^{13}C NMR chemical shifts (Table-1) of bellericagenin A were also found to be in conformity with its structure as 2α, 3β, 7α, 23-tetrahydroxyolean-12-en-28-oic acid (1). The ^{13}C signal assignments were straight forward using known chemical shift rules[13,14], off-resonance and DEPT studies[15].

The more polar acid, bellericagenin B (2), $C_{30}H_{48}O_7$ showed in its MS, the molecular ion peak at m/z 520. On treatment with CH_2N_2 it furnished a monomethyl ester (5) as shown by the ^{1}H NMR spectrum (3H, s, δ3.62). The ester (5), on acetylation, yielded a methyl ester tetraacetate (6) which displayed in its ^{1}H NMR spectrum four acetoxy methyl signals at δ1.98, 2.02, 2.05 and 2.11. The mass spectrum of the methyl ester tetraacetate (6) showed retro-Diels-Alder fragments suggesting the skeleton to be of Δ^{12}-oleanene or Δ^{12}-ursene[5]. The appearance of the fragment ions at m/z 425 $[a+H]^+$ and 278 (b') suggested that the four hydroxyl groups of bellericagenin B (2) are present in the part containing rings A/B, and that the carboxyl group and a free hydroxyl group are located in the part containing rings D/E. The result also disclosed that the methyl ester tetraacetate (6) contains a hydroxyl group which is hindered in nature as it was not acetylated under usual conditions. As the ^{13}C NMR spectrum (Table-1) of the acid (2) showed six quaternary carbons within the range 0-60 ppm its skeleton proved to be that of oleanane. The ^{1}H NMR spectrum of compound (6) exhibited two vicinal carbinyl protons at δ5.14 (1H, d, J 11 Hz) and 5.19 (1H, ddd, J 11, 11, 6 Hz) ascribed to H-3α and H-2β respectively. The spectrum also displayed two acetoxymethylene signals at δ 4.30 (2H, ABq, J 12 Hz), and 4.15 and 3.93 (1H each, d, J 12 Hz) assigned to 24-C$\underline{H}_2$OAc and 23-C$\underline{H}_2$OAc respectively. Compelling evidence for the presence of 23, 24-hydroxyls in bellericagenin B (2) was obtained as follows : The methyl ester (5), on treatment with trityl chloride[16] yielded the trityl derivative (7), which on acetylation furnished compound 8. On removal of the trityl groups of compound 8, the partially acetylated product 9 was obtained. The compound (9), on treatment with acetone and concentrated sulphuric acid[17] afforded the acetonide (10), suggesting the presence of primary hydroxyls in compound (2) at C-23 and C-24.

The location of the hindered hydroxyl group in the part containing rings D/E of bellericagenin B (2) was determined from the following evidences : Examination of the ^{13}C NMR assignments of a number of Δ^{12}-oleanenes[18,19] revealed that the lowest δ value (~30 ppm) of the six quaternary carbon signals, is assigned to C-20. In the ^{13}C NMR spectrum of compound (2), the singlet at ~δ 30 ppm was absent and instead a singlet appeared at δ35.5, suggesting a downfield shift of the peak presumably due to the presence of a hydroxyl function in the β-position i.e. either at C-21 or C-19. The magnitude of the chemical shift (δ80.9) ascribable to this carbinyl carbon disclosed the presence of a 19α-OH. The presence of the 19α-hydroxyl in bellericagenin B (2) was further confirmed by the ^{1}H NMR spectrum of the methyl ester tetraacetate (6) which displayed two signals at δ3.13 (1H, brs) and 3.33 (1H, t-like) attributable to H-18β and H-19β.

Thus the structure of bellericagenin B was defined as 2α, 3β, 19α, 23, 24-pentahydroxyolean-12-en-28-oic acid (2). The ^{13}C NMR data of bellericagenin B were also in good agreement with its structure (2) shown. Complete assignment of all the carbon chemical shifts of bellericagenin B (2) was accomplished by comparison with those of arjungenin[2] and belleric acid[2].

The neutral fraction of the $Bu^{n}OH$ soluble part on chromatographic purification over silica gel column yielded arjunglucoside I[2,4], bellericoside[2] and an intimate mixture of two compounds as revealed by TLC. This mixture on separation by repeated preparative TLC afforded two chromatographically pure compounds, both of which showed positive Liebermann-Burchard test for triterpenes and Molish test for sugars and thus appeared to be triterpenoid glycosides. The molecular weight of the less polar glycoside designated bellericaside A could successfully be determined to be 666 by liquid-secondary-ion mass spectrometry[20] (LSI-MS). The spectrum displayed ion peaks at m/z 689 and 705 formed by cationization of the molecule with Na^{+} and K^{+} respectively. Bellericaside A (11), $C_{36}H_{58}O_{11}$ showed in its IR spectrum a band at 1725 cm^{-1} suggesting the presence of an ester carbonyl function and thus indicated it to be an ester glycoside. The glycoside (11) on alkaline hydrolysis with 5% methanolic KOH (aq.) afforded a genin and a sugar constituent. The genin was found to be identical with bellericagenin A (1) (co-TLC and superimposable IR). The sugar was identified by paper chromatography as D-glucose by comparison with an authentic sample. The presence of D-glucose was also confirmed by GLC analysis after preparation of its alditol acetate. The attachment of D-glucose to the 28-COOH group of bellericagenin A (1) was evident from the ^{13}C NMR spectrum of the glycoside, bellericaside A (11). In the spectrum a signal at δ95.8 was assigned to C-1 of the glucose unit. Thus the structure of bellericaside A was established as β-D-glucopyranosyl 2α, 3β, 7α, 23-tetrahydroxyolean-12-en-28-oate (11). The ^{13}C NMR data (Table-1) of bellericaside A were also in good agreement with its structure (11) shown. Complete

Table 1. Chemical shifts [δ_C ($\pm$ 0.1)] of bellericagenin A **(1)**, bellericaside A **(11)**, bellericagenin B **(2)** and bellericaside B **(12)** (in C_5D_5N).

Carbon No.	(1)	(11)	(2)	(12)	Carbon No.	(1)	(11)	(2)	(12)
1	48.5	48.7	47.9	47.8	22	32.3	32.5	33.2[a]	33.0[a]
2	68.9	69.0	69.0	69.0	23	66.0	66.1	64.3	64.2
3	78.9	79.1	79.8	79.7	24	15.7	15.9	63.2	62.8
4	43.0	42.8	47.9	47.9	25	19.1[b]	19.0[a]	17.5[b]	17.5[b]
5	48.8	48.8	48.7	48.6	26	18.7[b]	18.8[a]	17.2[b]	17.2[b]
6	28.1[a]	28.2	19.5	19.4	27	26.3	26.1	24.9	24.8
7	67.4	67.6	33.3[a]	33.2[a]	28	180.1	176 .4	180.9	177.3
8	39.5	39.4	40.1	40.2	29	33.3	33.1	28.9	28.7
9	50.1	50.5	48.2	48.2	30	23.6	23.5	24.8	24.6
10	38.2	38.1	38.1	38.3	glc-1		95.8		
11	24.2	24.0	24.3	24.5	glc-2		74.0		
12	123.3	123.6	123.4	123.6	glc-3		78.3[b]		
13	144.1	143.5	144.8	144.3	glc-4		71.1		
14	41.9	41.8	41.9	42.0	glc-5		78.0[b]		
15	28.3[a]	28.2	29.0	28.9	glc-6		62.1		
16	23.8	23.6	28.0	27.9	gal-1				96.6
17	46.0	46.2	46.1	46.4	gal-2				71.7
18	44.7	44.5	44.9	44.5	gal-3				75.3
19	46.8	47.0	80.8	80.9	gal-4				69.2
20	31.0	30.7	35.6	35.5	gal-5				76.5
21	34.3	34.0	28.5	28.9	gal-6				60.5

glc = glucose, gal = galactose

a,b,c may be interchaged in each compound.

assignment of the ^{13}C NMR data of bellericaside A (11) was done by comparison with those of the aglycone, bellericagenin A (1) and methyl β-D-glucopyranoside using known chemical shift rules[13,14] and glycosylation shifts[21-24].

The molecular weight (682) of the more polar glycoside, bellericaside B (12) was determined to be 16 a.m.u. higher than that of bellericaside A (11) from the LSI-mass spectral study where the ion peaks at m/z 705 and 721 were attributed to $[M+Na]^+$ and $[M+K]^+$ respectively. Alkaline hydrolysis of bellericaside B (12) furnished bellericagenin B (2) and D-galactose, the latter being identified by PC and GLC. The attachment of D-galactose to the carboxylic acid group of bellericagenin B (12) was confirmed from the ^{13}C NMR spectrum of bellericaside B (12). Finally the structure was determined by assigning all the carbon chemical shifts of the glycoside, bellericaside B (12) by comparison with those of bellericagenin B (2) and methyl β-D-galactopyranoside using known chemical shift rules[13,14] and glycosylation shifts[21-24]. Consequently, the structure of bellericaside B was defined as β-D-galactopyranosyl 2α, 3β,19α,23,24-pentahydroxyolean-12-en-28-oate (12).

EXPERIMENTAL

All melting points were determined on a capillary melting point apparatus and are uncorrected. optical rotations were measured on a JASCO DPI-360 digital polarimeter. IR spectra were taken in KBr discs. ^1H NMR spectra were recorded either on a JEOL FX-100 (99.6 MHz) instrument or on a JEOL (399.8 MHz) spectrometer in $CDCl_3$. ^{13}C NMR spectra were taken on a JEOL (399.8 MHz) spectrometer operating at 100 MHz in C_5D_5N. Liquid secondary-ion mass spectra (LSI-MS) were obtained on a VG-XAB-SE mass spectrometer in nitrobenzyl alcohol (NBA) matrix with salt and without salt operating at 5-kV accelerating voltage, equipped with a 20-kV conversion dynode. Cesium ion was used as a bombarding particle. Electron impact mass spectra were recorded by direct inlet at 70 eV. TLC was carried out on silica gel G(BDH) plates using the solvent systems (A) $CHCl_3$-MeOH-H_2O (80:19:1) and (B) $CHCl_3$-MeOH-H_2O (35:13:2). The spots on the TLC plates were visualized by spraying Liebermann-Burchard reagent. 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 as staining agent. GLC was performed on a Hewlet-Packard model 5730A instrument using the column ECNSS-M, 3% on Gas Chrome Q at 190°C for the alditol acetates.

The air dried and powdered stem-bark of T. bellerica (3 kg) was first defatted with petroleum ether (60-80°C) and then exhaustively extracted with MeOH. The methanolic extract on removal of the solvent under reduced pressure yielded a viscous dark greenish brown mass (400g).

A part of this extract (250g) was partitioned between Bu^nOH and water. The organic layer was further washed with water for complete removal of inorganic impurities and free sugars. This Bu^nOH extract was separated into acidic and neutral fractions by treatment with a saturated solution of $NaHCO_3$.

Isolation of Bellericagenin A (1) and Bellericagenin B (2) - The acidic fraction after work up as usual gave a greenish residue (6.2g). The residue was chromatographed over a column of silica gel (130g) with petroleum ether, petroleum ether-$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$-MeOH (49:1, 24:1, 19:1 and 9:1) as successive eluents. The $CHCl_3$-MeOH (24:1) eluate (3.2g) on further separation yielded arjungenin[2,4] and belleric acid[2]. The $CHCl_3$-MeOH (19:1) eluate (1g) was subjected to preparative TLC using the solvent system (A) to give two chromatographically pure fractions. The fraction having a higher Rf value on repeated crystallization from MeOH furnished pure bellericagenin A (1) (300 mg), mp 297-298°C, $[\alpha]_D$+37.2° (c 0.25 in MeOH); IR $\nu_{max}^{cm^{-1}}$ 3450-3200 (hydroxyl), 1680 (acid carbonyl), 1635, 1060; EIMS m/z (rel. int.): 504 $[M]^+$(3), 486$[M-H_2O]^+$(6), 468$[M-2H_2O]^+$(7), 458$[M-HCO_2H]^+$(12), 441(9), 248(b)(78), 203(b-CO_2H)(96), 202(b-HCO_2H)(100), 189(93) and 187(99); ^{13}C NMR see Table-1 (Found : C, 71.34; H, 9.61; $C_{30}H_{48}O_6$ requires C, 71.39; H, 9.59%).

The fraction having lower Rf value afforded pure crystals (400mg) of bellericagenin B (2) from MeOH, mp>300°C, $[\alpha]_D$+33.4° (c 0.33 in MeOH); IR $\nu_{max}^{cm^{-1}}$ 3500-3300 (hydroxyl), 1675 (acid carbonyl), 1640, 1055; EIMS m/z (rel. int.): 520$[M]^+$(2), 502$[M-H_2O]^+$(5), 484$[M-2H_2O]^+$(3), 474$[M-HCO_2H]^+$(9), 457(15), 264(b)(63), 249(b-Me)(17), 246(b-H_2O)(90), 231(b-H_2O-Me)(25), 219(b-CO_2H)(20), 218(b-HCO_2H)(33), 201(b-CO_2H-H_2O)(100), 189(86) and 187(97); ^{13}C NMR see Table-1 (Found ; C, 69.22; H, 9.26; $C_{30}H_{48}O_7$ requires C, 69.20; H, 9.29%).

Bellericagenin A methyl ester (3) - Bellericagenin A (1, 100 mg) dissolved in MeOH (20 ml) was treated with an ethereal solution of CH_2N_2. The reaction mixture was kept overnight at 0°C and then worked up as usual to give a residue which on crystallization from MeOH yielded fine needles (70 mg) of bellericagenin A methyl ester (3), mp 218°C, $[\alpha]_D$+32.9 (c 0.29 in $CHCl_3$); IR $\nu_{max}^{cm^{-1}}$ 3500-3300 (hydroxyl), 1727 (ester carbonyl), 1638, 1380, 1260, 1160, 1060; ^1H NMR (99.6 MHz) δ0.89(3H, s), 0.96(3H,s), 1.00(3H,s), 1.08(3H,s), 1.27(3H,s), 1.45(3H,s)(together 6xMe), 3.61(3H,s, $COOCH_3$) and 5.35(t-like, H-12). (Found : C, 71.81; H,9.70; $C_{31}H_{50}O_6$ requires C, 71.78; H, 9.72%).

Bellericagenin A methyl ester triacetate (4) - Bellericagenin A methyl ester (3, 50 mg) was dissolved in pyridine (1 ml) and treated with Ac_2O (1 ml) at room temperature for 12h. After work up as usual, the product was purified by chromatography on silica gel followed by crystallization from MeOH to furnish microneedles (25 mg) of the triacetate (4), mp 171°C, $[\alpha]_D$+11.2 (c 0.25 in $CHCl_3$); IR $\nu_{max}^{cm^{-1}}$ 3650-3550 (hydroxyl), 1735, 1730, 1640, 1465, 1375, 1245, 1160, 1055; EIMS m/z (rel. int.):

644[M]$^+$(4), 626[M-H_2O]$^+$(1), 585[M-CO_2Me]$^+$(3), 584[M-AcOH]$^+$(5), 542[M-CO_2Me-COMe]$^+$ (1), 524[M-2AcOH]$^+$(3), 466(2), 451(2), 405(2), 262(b)(91), 247(b-Me)(6), 203(b-CO_2Me)(100), 202(b-AcOH)(25), 189(20) and 187(12); ^{1}H NMR (399.8 MHz) δ0.91(3H,s), 0.94(3H,s), 1.02(3H,s), 1.09(3H,s), 1.28(3H,s), 1.46(3H,s) (together 6xMe), 1.99(3H,s, OCOCH_3), 2.03(3H,s,OCOCH_3), 2.07(3H,s,OCOCH_3), 3.62(3H,s,COOCH_3), 3.74(1H,d,J 12Hz, H-23a), 3.95(1H,d,J 12Hz, H-23b), 4.35(1H,brs,H-7β), 5.01(1H,d,J 11Hz,H-3α), 5.23(1H,td,J 11, 6Hz, H-2β) and 5.34(1H,t-like, H-12). (Found : C, 68.87; H,8.77; $C_{37}H_{56}O_9$ requires C, 68.91; H, 8.75%).

Bellericagenin B methyl ester (5) - Bellericagenin B (2, 250 mg) was esterified with CH_2N_2 in the usual manner. The product (5) was crystallized from MeOH as microneedles (180 mg), mp 232°C, $[\alpha]_D$+29.1° (c 0.35 in $CHCl_3$); IR ν_{max} cm^{-1} 3550-3300 (hydroxyl), 1729(ester carbonyl), 1644, 1460, 1400, 1210, 1180, 1050; ^{1}H NMR (99.6 MHz) δ0.69(3H,s), 0.86(3H,s), 0.95(3H,s), 1.08(3H,s), 1.21(3H,s) (together 5xMe), 3.62(3H, s,COOCH_3) and 5.45(t-like, H-12). (Found : C, 69.65; H,9.42; $C_{31}H_{50}O_7$ requires C,69.63; H,9.43%).

Bellericagenin B methyl ester tetraacetate (6) - Compound (5, 60 mg) was acetylated with acetic anhydride and pyridine in the usual way. The acetate, thus obtained, on chromatographic purification followed by crystallization from EtOAc-hexane (3:1) mixture furnished fine leaflets of bellericagenin B methyl ester tetraacetate (6, 30 mg), mp 137°C,$[\alpha]_D$+8.3°(c 0.28 in $CHCl_3$); IR ν_{max} cm^{-1} 3600-3500 (hydroxl), 1750, 1648, 1460, 1375, 1230, 1160, 1050; EIMS m/z (rel. int.): 702[M]$^+$(4), 684[M-H_2O]$^+$(12), 643[M-CO_2Me]$^+$(6), 642[M-AcOH]$^+$(4), 624[M-AcOH-H_2O]$^+$(2), 582[M-2AcOH]$^+$(2), 463(3), 462(2), 451(3), 425[a+H]$^+$(3), 403(2), 383(3), 365[a-CO_2Me]$^+$(4), 305(2), 278(b')(70), 263(b'-Me)(6), 261(13), 260(b'-H_2O)(55), 245(21), 219(b'-CO_2Me)(12), 201(b'-H_2O-CO_2Me)(100), 189(8) and 187(15); ^{1}H NMR (399.8 MHz) δ0.67(3H,s), 0.85(3H,s), 0.96(3H,s), 1.09(3H,s), 1.22(3H,s) (together 5xMe), 1.98(3H,s,OCOCH_3), 2.02(3H,s, OCOCH_3), 2.05(3H,s,OCOCH_3), 2.11(3H,s,OCOCH_3), 3.13(1H,brs,H-18β), 3.33(1H,t-like, H-19β), 3.62(3H,s,COOCH_3), 3.93(1H,d,J 12Hz, H-23a), 4.15(1H,d,J 12Hz,H-23b), 4.30(2H,ABq,J 12Hz, 24-CH_2OAc), 5.14(1H,d,J 11Hz,H-3α), 5.19(1H,ddd,J 11,11,6 Hz, H-2β), 5.44(1H,t-like,H-12). (Found : C,66.66; H,8.30; $C_{39}H_{58}O_{11}$ requires C,66.64; H,8.32%).

Isopropylidene derivative (10) - To a solution of bellericagenin B methyl ester (5, 100 mg) in pyridine (4 ml), freshly crystallized and dried trityl chloride (400 mg) was added. The reaction mixture was kept at steam bath temperature for 20h. After cooling, pyridine (2 ml) and Ac_2O (3 ml) were added and the mixture was again heated at 100°C for 2h. After work up as usual, the product was hydrolysed with 80% AcOH (10 ml) for 3h. The residue obtained was dried *in vacuo* and treated with 10 ml of the reagent, Me_2CO-Et_2O-conc. H_2SO_4 (50:2:0.1) at room temperature for 24h. The reaction mixture on workup in the usual way yielded the acetonide (10) as

an amorphous powder, EIMS m/z 658. (Found : C,69.35; H,8.93; $C_{38}H_{58}O_9$ requires C, 69.27; H, 8.87%).

Isolation of Bellericaside A (11) and Bellericaside B (12) - The neutral fraction of the Bu^nOH soluble portion was evaporated to dryness under reduced pressure to give a viscous dark brown mass (65g). A part (40g) of this mass was chromatographed over a column of silica gel (1 kg). Graded elution was effected with petroleum ether, petroleum ether-$CHCl_3$(1:1), $CHCl_3$, $CHCl_3$-MeOH (19:1, 9:1, 22:3, 87:13, 43:7, 17:3 and 4:1). Fractions (250 ml each) were monitored by TLC. Fractions eluted with $CHCl_3$-MeOH (22:3) yielded previously reported[2] arjunglucoside I and bellericoside. The $CHCl_3$-MeOH (87:13 and 43:7) eluates (1.7g) were combined and subjected to preparative TLC separation on silica gel G plates with the solvent system (B). Thus bellericaside A (**11**, 200 mg) and bellericaside B (**12**, 300 mg) were isolated.

Bellericaside A (11) - Crystallized from MeOH as colourless powder, mp 207°C, $[\alpha]_D$ +17° (c 0.25 in MeOH); IR $\nu_{max}^{KBr}cm^{-1}$ 3550-3200 (hydroxyl), 1725, 1637, 1450, 1060; ^{13}C NMR see Table-1. (Found : C,64;81; H,8.81 ; $C_{36}H_{58}O_{11}$ requires C, 64.84; H, 8.77%).

Bellericaside B (12) - Crystallized from MeOH as a white amorphous powder, mp 223°C, $[\alpha]_D$+15.1°(c 0.30 in MeOH); IR $\nu_{max}^{KBr}cm^{-1}$ 3500-3200, 1729, 1637, 1461, 1398, 1070; ^{13}C NMR see Table-1. (Found : C,63.29; H,8.59; $C_{36}H_{58}O_{12}$ requires C, 63.32; H, 8.56%).

Alkaline hydrolysis of Bellericaside A (11) - Bellericaside A (**11**, 100 mg) was hydrolysed with 5% methanolic KOH (aq.) under reflux for 3h. The reaction mixture was acidified with dilute HCl and extracted with solvent ether. The ethereal layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue on chromatographic purification followed by crystallization from MeOH yielded bellericagenin A (**1**, 50 mg). The aqueous layer was passed through the column of Dowex 1-X2(OH^-) and 50W-X8(H^+) respectively and then concentrated under reduced pressure. The residue was tested for sugar by paper chromatography; D-glucose was identified by comparison with an authentic sample. The presence of D-glucose was also confirmed by GLC analysis after preparation of the alditol acetate.

Alkaline Hydrolysis of Bellericaside B (12) - Bellericaside B (**12**, 120 mg) was hydrolysed with 5% KOH-MeOH(aq.) and worked up as described above to yield D-galactose (identified by PC and GLC) and bellericagenin B (**12**, 60 mg).

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