Triterpene Sapogenins from *Primula denticulata* Sm.

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Summary: Five triterpenoid sapogenins named as pridentigenin A, B, C, D and E have been isolated after acid hydrolysis of the saponins from Primula denticulata. Structures have been suggested for the sapogenins.

Primula denticulata Sm (Primulaceae) occurs as a common weed in the mountains of North West Frontier province of Pakistan at a height of 7000 to 13000 feet.[1] A literature survey has revealed that no chemical investigation on the saponins and sapogenins of this plant has been carried out. In view of the medicinal properties attributed to many Primula species,[2,3] we undertook the study of the saponins and sapogenins of *Primula denticulata*. The isolation and structure elucidation of three of its sapogenins were reported by us in preliminary communications[4,5,6] The present paper describes the experimental procedure in detail.

The alcoholic extract of the whole plant was evaporated, worked up and hydrolysed as described in the experimental part. Thin layer chromatography of the hydrolysed genin mixture showed several triterpenoid constituents, out of which five could be isolated in pure state through column chromatography on alumina and subsequent preparative layer chomatography. They were named as pridentigenin A, pridentigenin B, pridentigenin C, pridentigenin D and pridentigenin E in increasing order of polarity on t.l.c. plate.

Pridentigenin A (Ia)

Pridentigenin A is the most nonpolar sapogenin in this plant. It crystallises from ether in the form of colourless needles m.p. 308-10°. In the infrared spectrum ($CHCl_3$) it shows a sharp band at 3520 cm^{-1} (OH) and a strong peak at 1700 cm^{-1} due to carbonyl group. There is no absorption between 1600-1690cm^{-1} indicating the absence of C=C stretching.

The nuclear magnetic resonance spectrum ($CDCl_3$) of pridentigenin A shows the presence of six tertiary methyl groups through singlets at δ 0.70 (3H, 1 x CH_3), 0.84 (6H, 2 x CH_3), 0.97 (3H, 1 x CH_3), 1.03 (3H. 1x CH_3), 1.24 (3H, 1 x CH_3). There is a singlet equivalent to six protons at δ 3.51 indicating the presence of two magnetically equivalent OCH_3 groups in the compound. Another singlet at δ 4.30 is ascribed to H-30.

In the mass spectrum, the molecular ion peak is visible at m/e 516 corresponding to the molecular formula $C_{32}H_{52}O_5$. Other important peaks were observed at m/e 485 (base peak, M-OCH_3): 484 (M^+ – CH_3OH), 466 (M^+ – (CH_3OH + H_2O), 454 (M^+ –(OCH_3 + CH_2OH), 452(M^+ – $2CH_3OH$), 308 (retro-Diels Alder fragment a)[7], 277 (a-OCH_3) 276 (a–CH_3OH), 207 (retro-Diels – Alder, fragment b), 189 (b–H_2O).

On acetylation with acetic anhydride and pyridine at room temperature, pridentigenin A yielded a monoacetate (Ib), m.p. 270-72°. The infrared spectrum ($CHCl_3$) of pridentigenin A monoacetate shows no absorption between 3000-3600 cm^{-1} indicating the absence of hydroxyl group in this derivative. A strong band at 1735 cm^{-1} is due to presence of acetate groups and another band at 1707 cm^{-1} shows the presence of carbonyl group in the molecule.

The mass spectrum of acetyl pridentigenin A exhibited molecular ion peak at m/e 558 showing the

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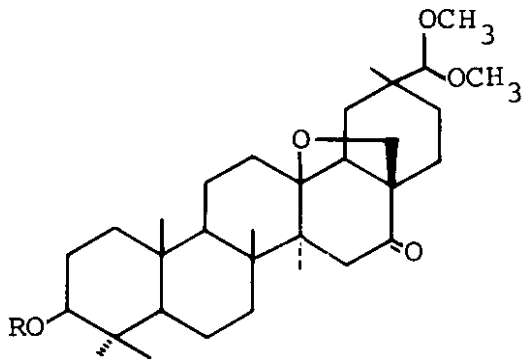
shift of 42 units from the molecular peak of the compound. This confirms that there is only one hydroxyl group in pridentigenin A. There is no double bond in pridentigenin A, but its fragmentation pattern behaves like other triterpenoids having double bond at C(12)-C(13). Other mass fragmentation peaks are also shifted by 42 units.

It is clear from the above spectral data that out of five oxygen atoms in pridentigenin A, one is present as hydroxyl group, one as a carbonyl group and two as a O-methyl groups. The fifth oxygen atom is probably present as an ether function. The hydroxyl group is apparently present in ring A or B as indicated by a peak at m/e 207 in pridentigenin A is shifted to 249 in its acetate corresponding to moiety b. A peak at m/e 189 in both pridentigenin A as well as in its acetate correspond to elimination fragment b. It is very likely from biogenetic view point that this hydroxyl group is present at C-3 with a β configuration. The presence of two methoxy groups indicated in the NMR spectrum is also confirmed by the loss of two molecules of methanol in the mass spectra of pridentigenin A and its acetate resulting in peaks at m/e 452 and 494.

On surveying the literature it was found that the spectral and other physical data of pridentigenin A closely resemble with that of cyclamigenin D reported by Dorchai et al[8]. It is therefore very likely that the two compounds are identical. Unfortunately no authentic sample of cyclamigenin D was available for direct comparison.



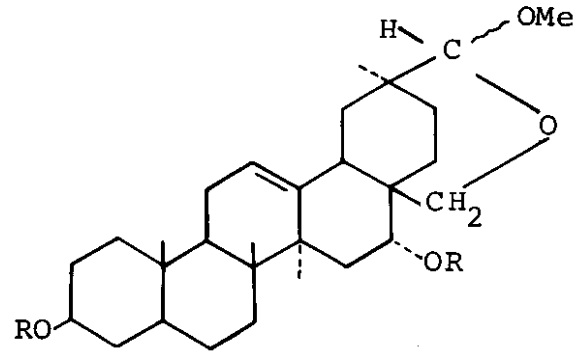
(I) a, R=H

b, R=Ac

Pridentigenin B(IIa)

Pridentigenin B analyses for $C_{31}H_{50}O_4$. Its UV spectrum has λmax at 212-213 nm in methanol which shows the absence of conjugated double bonds in the compound. In the i.r. spectrum (KBr) a strong peak at 3367 cm^{-1} (OH) and a peak of medium intensity at 1630 cm^{-1} (C=C stretching) are visible. No carbonyl bands are present. The mass spectrum of pridentigenin B shows a very low intensity molecular ion peak at m/e 486 which becomes somewhat stronger when the field desorption technique is used. Other peaks were found at m/e 468 ($M^+ - H_2O$), 455 ($M^+ - OCH_3$), 454 ($M^+ - CH_3OH$), 357 ($C_{24}H_{37}O_2$) formed by cleavage of rings E), 278(fragment a formed in the retro-Diels Alder cleavage), 246 (a–CH_3OH), 207 ($C_{14}H_{23}O$), fragment b in the same cleavage). The last peak indicates that there is only one oxygen atom in ring A ro B and the peak at 189(a-H_2O) shows that this is in the form of hydroxyl group. (Scheme 1).

On the treatment with acetic anhydride and pyridine, pridentigenin B forms a diacetate (II b) m.p. 222°C.

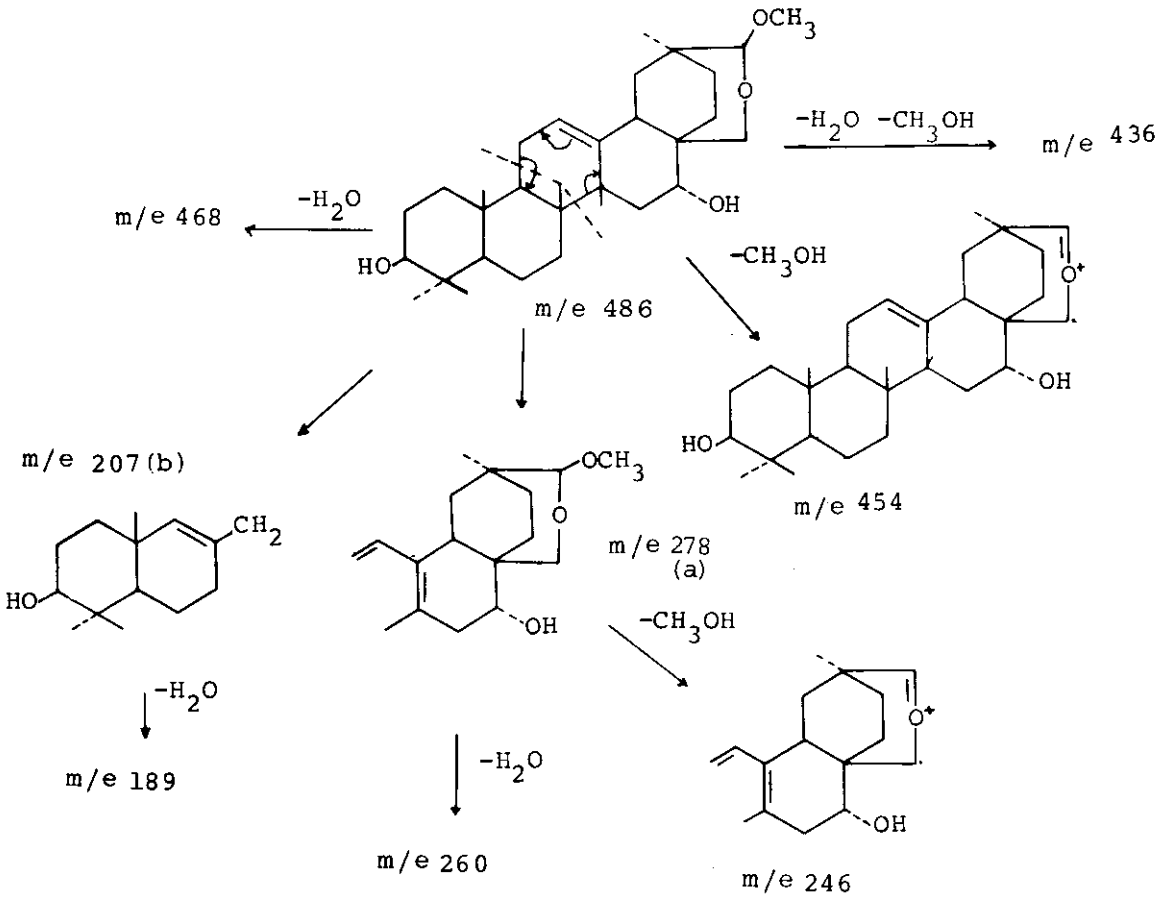


(II) a, R=H

b, R=Ac

Diacetyl pridentigenin B shows a strong peak at 1745 cm^{-1} in the i.r. spectrum ($CHCl_3$) due to acetyl groups but no absorption which could be assigned to hydroxyl groups. This clearly shows that out of the four oxygen atoms in pridentigenin B only two are present as hydroxyl groups and the other two as ether groups, where as no carbonyl functions are present. Out of these two hydroxyls, one is present in ring A or B as stated above, most probably at C-3 from the biogenetic point of view.

The ^{1}H-nmr spectrum of diacetyl-pridentigenin B shows four singlets due to 6 tertiary methyl groups at δ 0.87 6H, 2xCH_3, 0.92 (6H, 2xCH_3), 0.93 (3H, 1xCH_3), 1.24 (3H, 1xCH_3). The position of the last singlet is due to C(14)–CH_3 in accordance with

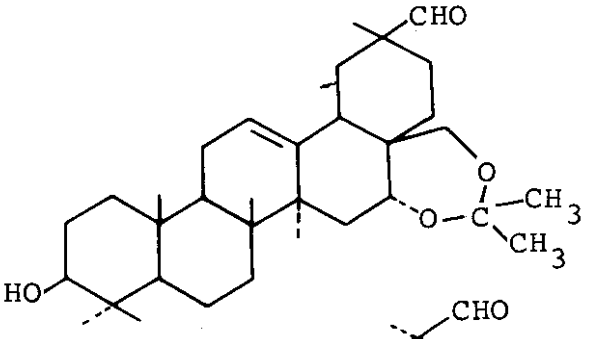


Scheme 1: Fragmentation pattern of Pridentigenin B

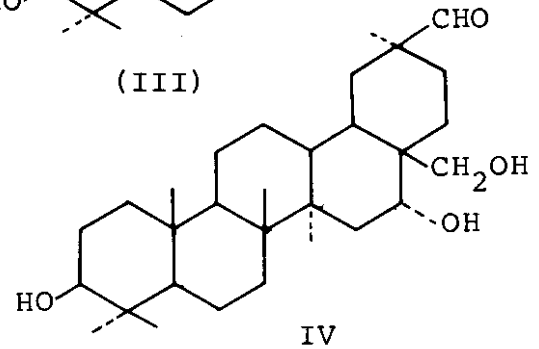
the OH at C–16[9]. There are 2 singlets at δ 2.00 and 2.05, represents two o-acetyl gtoups, a singlet at δ 3.36 exhibits OCH_3 group. The C(28)H_2 double doublet was centred at δ 3.51 (2H, J=20Hz). In addition there is a singlet at δ 4.25 C(30)–H, a multiplet between δ4.30 and 4.61 which is due to C(3)–H and C(16)–H. This multiplet occurs at a higher field in the unacetylated product and is therefore assigned to protons attached to carbon atoms bearing OAc groups. The triplet like signal due to C(12)–H occurs, as expected, at δ 5.34.

The mass spectrum of diacetyl pridentigenin B shows a very small M^+ peak at m/e 570 but a very strong ($M^+ - CH_3OH$) peak at m/e 538. Peaks at m/e 510 (M^+. $-CH_3COOH$), 478 ($M^+ -(CH_3OH + CH_3COOH)$, 288 (retro-Diels-Alder-fragement of a-CH_3OH ion), 189 (retro-Diels Alder fragment of b-CH_3 COOH ion) are also visible. On treatment of pridentigenin B with acetone in the presence of anhydrous copper sulphate, an acetonide (III) is formed, but only with difficulty and incompletely. This happens apparently with the opening of the acetal ring and elimination of the methoxyl group as methanol. This is demonstrated by a strong aldehyde peak at 1720 cm^{-1} in the i.r. spectrum as well as mass spectral peak at m/e 512 (M^+), 497 (M-CH_3). The formation of acetonide between the C(28) H_2OH and a C(16) OH groups is already known[10]. The acetal group in pridentigenin B was confirmed by mild acidic hydrolysis to the free aldehyde. The hydrolysis of the sapogenin (or its diacetyl derivative) in dilute tetrahydrofuran containing catalytic amount of HCl did not yield satisfactory results. The hydrolysis was therefore carried out in the MeOH-H_2-HCl mixture and the product was separated from the starting material through preparative layer chromatography. The hydrolysis product (m.p. 240°) was identified with cyclamiretin D(IV) lit. M.p. 242°) through spectroscopic studies, as unfortunately no authentic sample was availabe for direct comparision. It showed a peak at 1710 cm^{-1} in the i.r. spectrum due to the aldehyde group, in addition to the hy-

droxyl absorption at 3440-3480 cm^{-1}. The n.m.r. spectrum revealed methyl singlets at δ 0.79 (3H, 1 x CH_3), 0.92 (3H, 1 x CH_3), 1.00 (6H, 2 x CH_3) 1.38 (3H, 1 x CH_3), 1.59 (3H, 1 x CH_3). The last signal is assigned to C(29)H_3, and its relatively down-field position is due to its attachment to C(20) bearing the aldehyde group. This is confirmed by its upfield shift in the tetraacetate of the borohydride reduction product. In addition there were peaks at δ 3.27 (broad singlet, C(28)H_2 OH) 4.08 (multiplet, C(16) HOH), δ 5.33 C(12)H and 8.46 (CHO). The mass spectrum was similar to that of cyclamiretin D published by Tschesche (1oc. cit).



(III)



IV

On the basis of the above findings we propose that pridentigenin B has the structure (IIa). It is therefore a homologue of cyclamigenin A (or C) isolated by Dorchai et al[8]. The stereochemistry at C-30 could not be determined with certainty but it is likely that it is the thermodynamically more stable equatorial 30β epimer. The structure proposed for pridentigenin B finds a measure of support from the ^{13}C n.m.r. spectrum in $CDCl_3$ + CD_3OH. Thus in analogy with the spectra of olean-12-enes[11] we find peaks due to C(12) and C(13) at 121.5 and 142.1 ppm. The C(3) and C(16) bearing the hydroxyl groups show peaks at 78.93 and 77.43 ppm respectively. Whereas the peaks of C(28) attached to an ether function occurs at 70.1 ppm. The peak due to C(30) which is attached to two oxygen atoms is shifted to 109.7 ppm. The assignment of other ^{13}C-n.m.r peaks has already been reported in the earlier communication.[4]

Pridentigenin C(II a)

Pridentigenin C was isolated from the middle fractions eluted with chloroform:methanol (90:10 v/v) along with pridentigenin D. It was further purified through preparative layer chromatography using chloroform: methanol.(9:1 v/v) as developing system. Pridentigenin C was crystalised from methanol into colourless crystals m.p. 152-154°.

The nuclear magnetic resonance spectrum of pridentigenin C ($CDC1_3$) shows the presence of six methyl groups at δ 0.69 (3H, 1 x CH_3), 0.81 (6H, 2 x CH_3), 0.87 (6H, 1 x CH_3) 1.21 (3H, 1 x CH_3). Whereas methoxy group appeared at δ 3.51 (3H, OCH_3), a proton appearing in ring at δ 4.13 (1H) is attached at C-30, another broad signal at δ 5.34 is due to proton at C-12.

The mass spectrum of pridentigenin C shows a very weak molecular ion peak at m/e 486. Fragment at m/e 278 and 207 shows that this molecule undergoes retro Diel's Alder fragmentation pattern. Fragmentation pattern and relative intensities of the peaks are given in the experimental part.

The NMR spectrum and mass fragmentation pattern of pridentigenin C is very similar to that of pridentigenin B. It appears that pridentigenin C is a stereoisomer of pridentigenin B. The two compounds show difference in chromatographic behaviour, pridentigenin C being more polar. Pridentigenin C is probably the less stable epimer of pridentigenin B, as it is obtained in lower yield. Attempts are being made to isolate pridentigenin C in larger quantities for more complete characterization of the compound.

Pridentigenin D (Va)

Pridentigenin D was isolated from the middle fraction eluted with chloroform:methanol (9:1 v/v) and subsequently purified through preparative layer chromatography. Pridentigenin D was crystallized from ether into small colourless crystals m.p. 170°

The infrared spectrum shows a broad band at 3360 cm^{-1} due to OH group, a sharp peak at 1700 cm^{-1} due to carbonyl function and a medium intensity peak at 1612 cm^{-1} due to C=C stretching. The nuclear magnetic resonance spectrum (400 MHz) of Pridentigenin D ($CDC1_3$) shows five singlets at δ 0.78 (3 H, s, 24-Me) 0.88 (3H, s, 26-Me), 0.94 (3H, s,

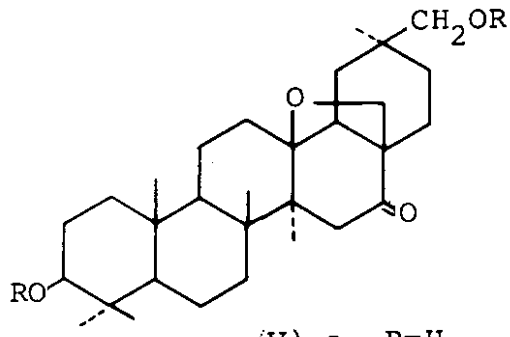
25-Me), 0.97 (3H, s, 29-Me), 1.05, (3H, s, 23-Me), and 1.23(3H, s, 27-Me) representing six methyl groups. The double doublet at δ 3.19 (J=11.5Hz, 5.5H_z) is due to H-3 α and therefore the compound has a 3β-OH in it. There are two doublets at δ 2.14 and 2.71 (J=16 Hz) assigned to the two H-15 protons in the vicinity of carbonyl group at C-16, the higher field doublet shows a further long range coupling (J=2 Hz) with a methyl group and each wing is split up into a quartet. A double doublet at δ 3.47 and 3.87 (J=8 Hz each) is assigned to the two H-30 protons whereas the two H-30 protons also appear as double doublets at δ 3.32 and 3.58 (J=11 Hz each). The fact that the two H-30 protons are magnetically non equivalent indicates a hindered rotation of the H(20) –H(30) bond. Dreiding model shows that the hindrance in rotation is possible only if the OH group is attached to C-30 and not C-29. There is no signal due to the olefinic proton.

The mass spectrum shows a medium intensity molecular ion peak at m/e 472.35097 corresponding to molecular formula $C_{30}H_{48}O_4$ calc. 472.35520 In addition strong peaks are present at m/e 455(M^+ – OH), 454 (M^+–H_2O), 442(M^+ –CH_2O) 441 (M^+ – CH_2OH), 423 (M^+ –(H_2O + CH_2OH), 411 (M^+ – (CH_2OH + CH_2O), 357, 264 (100%, c, Retero Diel Alder's fragment[7] a), 251, 246(a-H_2O), 233 (a-CH_2OH), 219, 215 (a-H_2O + CH_2OH), 207 (Retero Dield Alder's fragment b), 189 (b–H_2O). The peaks at m/e 207 and 189 indicate that the carbonyl group is not located in ring A or B. There is only one hydroxyl group in these rings, probably a β C(3) –OH from biogenic point of view. The other three oxygen atoms are present in rings D & E.

On acetylation with acetic anhydride and pyridine at room temperature, pridentigenin D afforded a diacetate. (Vb).

Its infrared spectrum shows no absorption between 3300-3600 cm^{-1} indicating the absence of hydroxyl group. Two prominent bands at 1715 cm^{-1} and 1700 cm^{-1} are due to OAc and carbonyl absorption respectively, A medium intensity peak at 1600 cm^{-1} indicates C=C stretching in the molecule.

The mass spectrum of the diacetate shows a molecular peak at m/e 556. Other peaks were present at m/e 496 (M^+–CH_3COOH), 454, 412, 306(c), 293 (d), 275(f), 261(i), 229, 215, 203(g), 190, 189, (e), 175, 147(h). The presence of the carbonyl groups in ring D is confirmed by the presence of a peak at m/e 147 (h) (scheme 2.) On the basis of the above spectral data we propose the structure (V) for the new sapogenin pridentigenin D. This structure finds a measure of support from the biogenic point of view because other compounds containing closely related structures have been isolated by us from this plant.



(V) a, R=H

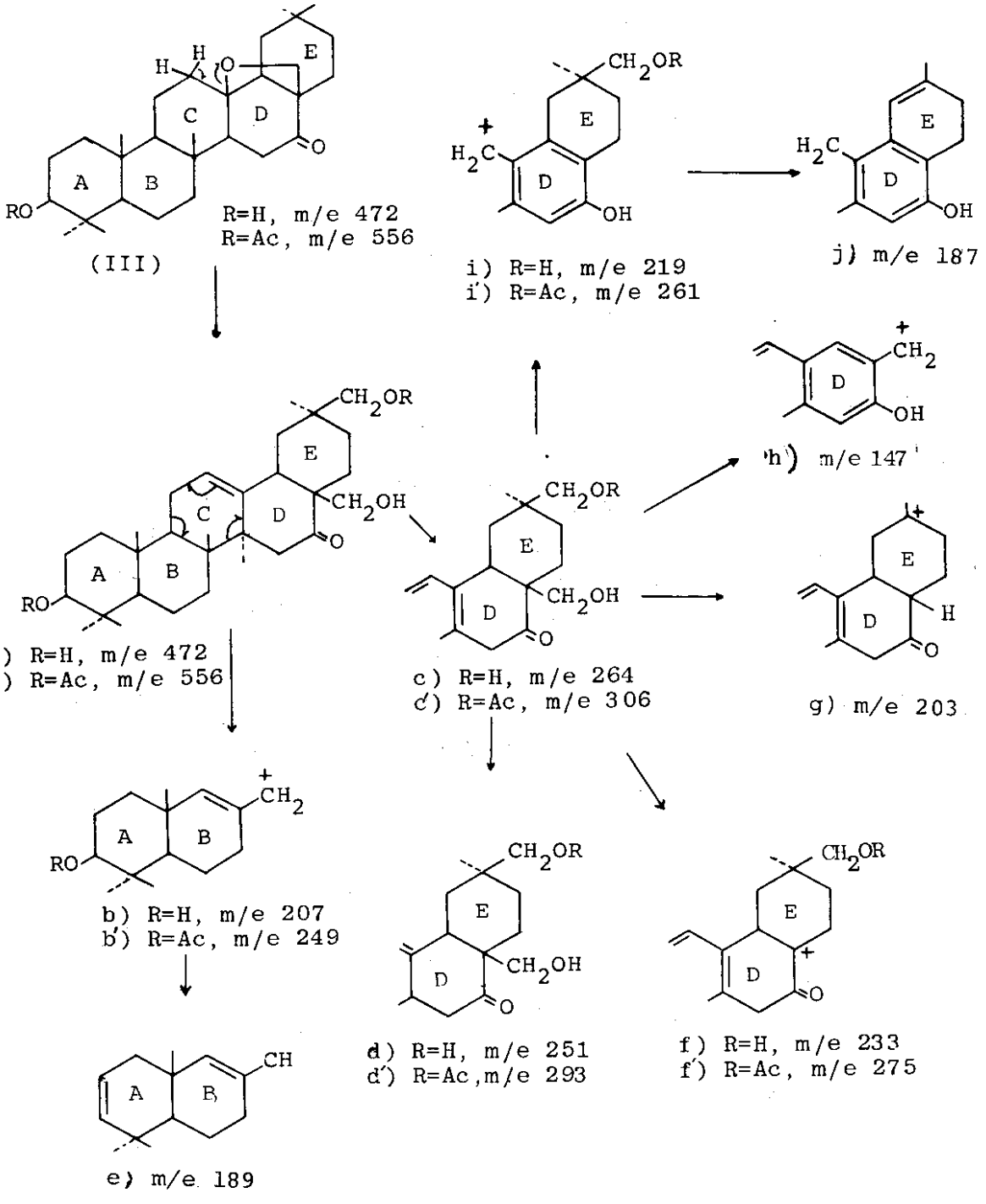
b, R=Ac

Pridentigenin E (VI a)

The fractions eluted with chloroform:methanol (8:2 v/v) and pure methanol revealed the presence of one major component with some more polar and some less polar impurities. From these combined fractions pridentigenin E was separated through preparative thick layer chromatography on sillica gel G and crystalized from methanol. The purity of the compound was checked on t.l.c. plate. After recrystalization it melted at 268-270° and analysed for $C_{30}H_{50}O_4$.

The ultraviolet spectrum of the pridentigenin E in methanol shows only an absorption band at λmax 212 nm. Its infrared spectrum (KBr) shows a broad intense peak at 3340 cm^{-1} indicative of the presence of hydroxyl group and a medium size peak at 1635 cm^{-1} due to olefenic double bond stretching vibration, a peak at 1000 cm^{-1} shows the presence of C-O group, and at 820 cm^{-1} characteristic of double bond at C(12)–C(13) carbon in pentacyclio triterpenes. There is no carbonyl absorption.

The nuclear magnetic resonance of pridentigenin E spectrum in C_5D_5N shows four signals in upper field which corresponded to six methyl groups at δ 1.08 (6H, 2 x CH_3), 1.25 (3H, 1 x CH_3), 1.31 3H, 1 x CH_3) and 1.84 (6H, 2 x CH_3) two signals at δ 3.71 and 3.97 corresponded to four protons due to two CH_2OH, the later signal has been assigned to functional group at C-28, a broad signal at δ 6.64 is due to vinyl proton at C-12.



Scheme 2: Mass Spectrometric fragmentation of Pridentigenin D

The mass spectrum of pridentigenin E shows a low intensity molecular ion peak at m/e 474. The mass spectral fragmentation peaks and relative intensity of the observed peaks are given in the experimental part.

The pridentigenin E was acetylated with acetic anhydride and pyridine. The acetylated product of pridentigenin E (VI b) could not however be obtained in a crystalline form but its purity was established through t.l.c. The infrared spectrum of the acetylated product shows no absorption in the region 3300-3600 cm^{-1} indicating the absence of hydroxyl groups, a strong absorption at 1720 cm^{-1} and 1710 cm^{-1} is due to O-acetyl groups. the nuclear magnetic resonance spectra ($CDCl_3$ 100 MHz) of the acetylated pridentigenin E shows four signals corresponding to six methyl groups at δ 0.81 (6H, 23-Me, 24 – Me), 0.90 (3H, 26-Me), 0.93 (25-Me, 29-Me), 1.25 (3H, 27-Me). There are four singlets at δ 2.03 (3H), 2.04 (3H), 2.06 (3H), and 2.07 (3H) due to four O-acetyl groups, a triplet appearing at δ 4.48 is attributed to α-3-H, whereas two singlets at δ 5.10 and 5.30 represents the protons at C-16 and C-12 (vinyl proton) respectively. A quartet centered at δ 3.87 (4H, J_{AB}=7.2 Hz) is due to AB coupling assignable to methylene protons at C-28 and C-30.

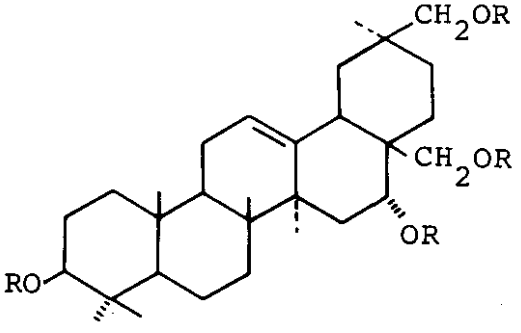
In the mass spectrum molecular ion peak is absent, a peak at m/e 582 is due to the elimination of molecule of acetic acid from the molecular peak (M^+–60). The mass spectral fragmentation and relative intensity of the fragments are given in experimental part.

In view of NMR spectral data and mass fragmentation pattern it is concluded that pridentigenin E is a pentacyclic triterpenoid. The NMR spectra shows six methyl groups without coupling and mass spectral fragmentation pattern in which fragment ion at m/e 203 is stronger than the peak at m/e 191 shows that pridentigenin E is a β-amyrin type of triterpenoid.[7]

Treatment of pridentigenin E with acetone in the presence of anhydrous copper sulphate furnished a less polar product acetonide in a small yield which was purified through preparative layer chromatography. The infrared spectrum of the acetonide (VII) shows a band at 3400 cm^{-1} indicating the presence of hydroxyl group. The mass spectrum of pridentigenin E acetonide shows a molecular ion peak of low intensity at m/e 514. The mass spectral fragmentation pattern and relative intensity of the peaks are given in the experimental part.

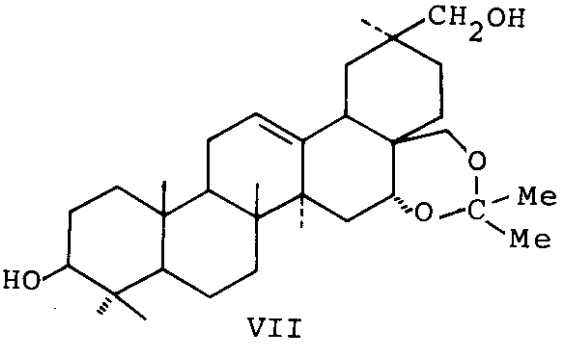
On the basis of the above spectra, it is suggested that pridentigenin E is identical to dihydrocyclamiretin D. This compound was first prepared by Barton et al,[12] later by Tschesche[13] through the reduction of their cyclamiretin by borohydride. The compound has been isolated by Ito et al[14] from *Cyclamen europea* and given the trivial name of cyclamiretine. Since an authentic sample of dihydrocyclamiretin D could not be obtained, it was prepared through the borohydride reduction of cyclamiretin D (IV) which was obtained through the hydrolysis of pridentigenin B. The identity of pridentigenin E with the dihydrocyclamiretin D so obtained was proved through mixed melting point, t.l.c. and suprimposable IR spectra of the two samples. Therefore pridentigenin E has the structure: VI a.

Since methanol was used as solvent in acidic hydrolysis of saponins and crystallization of sapogenins, it is possible that the pridentigenin A,B and C, containing a methyl acetate group are artefacts. The work on isolation of saponins from *Primula denticulata* in pure state is in progress.



(VI) a, R=H

b, R=Ac



VII

EXPERIMENTAL

Primula denticulata (3.5 Kg) was collected from Dongagali (North Western Frontier Province of Pakistan), Shade dried, then ground to coarse powder and extracted with MeOH under reflux. The residue was shaken with n-BuOH and water and the n-BuoH layer was evaporated. The residue was dissolved in minimum quantity of MeOH and was diluted with cold Et_2O yielding a cream coloured precipitate of crude saponin (200 gm.) 100 gm of the saponins were taken up in 1N methanolic hydrogen chloride and refluxed for about 5 hours in hot water bath. After the hydrolysis was complete, the reaction mixture was diluted with water, furnishing a crude precipitate containing sapogenins (35g). Thin layer chromatography of sapogenin mixture with $CHCl_3$ – MeOH (9:1– showed, after spraying with ceric sulphate reagent (1 % ceric sulphate in 10% H_2SO_4) and subsequent heating, five components which were assigned the names as pridentigenin A,B,C,D,E in increasing order of polarity on the plate along with some minor components.

The mixture of sapogenin was chromatographed on neutral alumina and was eluted successively with C_6H_6, C_6H_6– $CHCl_3$, (9:1, 8:2, 1:1), $CHCl_3$, $CHCl_3$– MeOH (9:1, 8:2) and with pure MeOH.

PRIDENTIGENIN A

Pridentigenin A was isolated from the mother liquor of Pridentigenin B through preparative thick layer chromatography using $CHCl_3$–MeOH (9.7:0.5 v/v) as developing solvent. Crystallzation of the pridentigenin A into fine crystals was achieved from Et_2O, m.p. 308-310°

IR($CHCl_3$): 3520 (Sharp, OH), 1700 cm^{-1} (strong, carbonyl group). no absorption between 1600-1690 cm^{-1}.

NMR($CDCl_3$): δ 0.79 (3H, 1 x CH_3), 0.84 (6H, 2 x CH_3), 0.97 (3H, 1 x CH_3), 1.03 (3H, 1 x CH_3), 1.24 (3H, 1 x CH_3), a singlet at δ 3.51 (6H, 2 x OCH_3), 4.30 (1H, C-30).

Mass: m/e 516(M^+, 20.19%), 485(100.00%), 484 (47.11%), 466(16.34%), 454(48.07%), 452 (57.69%), 308(Fragment a, 69.23%), 277 (17.30%), 276(19.23%), 207(Fragment b, 57.69%), 189(47.11%) 75(100.00%).

PRIDENTIGENIN A MONOACETATE

Pridentigenin A monoacetate was prepared by dissolving about 20mg of the pridentigenin A in Ac_2O, to which a few drops of pyridine were added. The reaction mixture was kept at room temperature over night. Ice was added to the reaction mixture which gave white precipitate. The precipitate was filtered and dried m.p. 270-272°.

IR(KBr): 1735(OAc) and 1707 cm^{-1} (carbonyl).

Mass: m/e 558(M^+, 20.90%), 526(97.27%), 494 (54.54%), 466(25.45%), 308(Fragment a, 50.00%), 277 (27.27%), 263 (55.45%), 249 (Fragment b, 29.00%), 189 (69.00%), 75 (100.00%).

PRIDENTIGENIN B

The fraction eluted with C_6H_6, C_6H_6–$CHCl_3$ (9:1, 8:2) yield white crystals on evaporation, which were then recrystallized from MeOH into white thin needles of pridentigenin B m.p. 220-222°.

Pridentigenin B analysis for : $C_{31}H_{50}O_4$
Found: C, 76.22 H, 10.10%
Calculated for $C_{31}H_{50}O_4$, C, 76.54, H, 10.28%.

UV (methanol): λ_{max} 212-213 nm.
IR(KBr): 3367 by (OH), 1630 cm^{-1} (C=C) 2460, 1388 1355, 1260, 1100, 980 and 825 cm^{-1}.

NMR($CDCl_3$) δ 0.8 (3H, 1 x CH_3), 0.87 (3H, 1 x CH_3) 0.90(6H, 2 x CH_3) 1.03 (3H, 1 x CH_3), 1.24 (3H, 1 x CH_3), 3.41(3H, OCH_3), 5.34 (1H, C-12 (H), 4.25 (1H, C-30(H)).

Mass: m/e 486(M^+, 0.73%) 468(1.47%), 455(6.2%), (100.00"), 436, (6.61%), 424(7.35%), 379 (4.28%), 357 (80.88%), 278 (Fragment a, 5.88%), 260(7.35%), 246(44.11%), 234(31.61%), 216 (30.80%), 207(Fragment b, 59.55%), 189 (42.64).

PRIDENTIGENIN B DIACETATE

Pridentigenin B (100 mg) was dissolved in one ml of Ac_2O with slight warming and a few drops of pyridine were added. The reaction mixture was left overnight at room temperature. Ice was added to the

reaction mixture which furnished white precipitate. It was filtered and dried. The acetylated products was crystallized from MeOH yielding fine colourless crystals m.p. 222°. Thin layer chromatography in $CHCl_3$ as solvent system of the crystals on silica gel G showed a single spot less polar than the starting material, which showed that the acetyl product is pure.

IR ($CHCl_3$): 1745 cm^{-1} (O-acetyl) 2460, 1388, 1355, 1260, 1100, 980 and 825 cm^{-1}.

NMR($CDCl_3$): δ 0.87 (6H, 2 x CH_3), 0.92 (6H, 2 x CH_3), 0.93 (3H, 1 x CH_3), 1.24 (3H, 1 x CH_3), 2.00 (3H, OAc) and 2.05 (3H, OAc), 3.36 (3H, OCH_3), 4.25 (1H, C-30(H)), 4.3-4.61(2H, C-3H, and c-16H) 3.51 (dd, 2H, j= 20Hz), 5.34 (t-like, C-12H).

Mass: m/e 570(M^+–CH_3COOH, 1.45%), 538(100%), 510 (6.90%), 478 (10.54%), 320 (Fragment a, 0.72%), 288 (27.77%), 260(19.27%), 249(Fragment b, 2.08%), 228(37.45%), 189 (19.63%).

ACETONIDE OF PRIDENTIGENIN B

Pridentigenin B (20 mg) was dissolved in anhydrous Me_2CO to which anhydrous copper sulphate was added. The reacting material was stirred for 36 hours at room temperature and then filtered. Thin layer chromatography of the filtrate on silica gel G plate showed two spots in $CHCl_3$: MeOH (9.5:0.5 v/v) as developing solvent followed by spraying with ceric sulphate reagent. The polar spot corresponded with the starting material. The non-polar component was separated through preparative layer chromatography which was found chromatographically pure. It was obtained in the form of colourless powder.

IR($CHCl_3$): 3220 cm^{-1} (OH), 1720 cm^{-1} (CHO) 1600 cm^{-1} (C=C)

Mass: m/e 512(M^+, 13.95%), 497(15.89%), 454 (17.44%), 439 (33.33%), 304 (Fragment a, 100%), 289 (20.54%), 246(21.70%), 231 (20.15%), 217 (20.15%), 207 (Fragment b, 60.07%), 189). (10.17).

ACID HYDROLYSIS OF PRIDENTIGENIN B

Pridentigenin B (20 mg) was dissolved in MeOH diluted with water and acidified with dilute HCl and stirred for two days at room temperature. Satisfactory results were achieved by adding one to two drops of water from time to time. Thin layer chromatography of the hydrolysed mixture on silica gel G plate developed in $CHCl_3$: MeOH (9:1 v/v) showed two components, the non-polar being the starting material and polar being the hydrolysed product. The hydrolysed product was separated through preparative layer chromatography using $CHCl_3$: MeOH (9:1 v/v) as developing system. The isolated compound mp. 240° and gave positive test for carbonyl group with 2-4 dinitrophenylhydrazine reagent.

IR($CHCl_3$): 3440-3480 cm^{-1} (OH), 1720 cm^{-1} (carbonyl).

NMR($CDCl_3$): at δ 0.79 (3H, 1 x CH_3), 0.92 (3H, 2 x CH_3), 1.00 (6H, 2 x CH_3), 1.38 (3H 1 x CH_3), 1.59(3H, 1 x CH_3), 3.27 (bs, C-28 (H_2-OH)), 4.08 C-16(H–) H)), 5.33 (C-12 (H)), 8.46 (CHO).

Mass: m/e 472(M^+, 0.31%), 454 (78.57%), 441 (2.23%), 425(34.84%), 394 (6.25%), 264 (Fragment a, 27.23%), 246 (42.41%), 235(100%) 215(56.44%), 207(72.32%), 189(46.87%).

PRIDENTIGENIN C

Pridentigenin C was isolated from the middle fraction eluted with $CHCl_3$: MeOH (90:10 v/v) along with pridentigenin D. It was then further purified through preprative thick layer chromatography using $CHCl_3$: MeOH 1(9:1 v/v) as developing system. The bands were visualized on spraying with water which were then scratched out and eluted with $CHCl_3$: MeOH (mixture). Solvent from the eluent was evaporated and the residue crystallized from MeOH where large colourless crystals of pridentigenin C, m.p. 152-154° were obtained.

NMR($CDCl_3$) δ 0.69 (3H, 1 x CH_3) 0,81 (6H, 2 x CH_3), 0.87 (6H, 2 x CH_3), 1.21 (3H, 1 x CH_3), 3.51 (3H, s, OCH_3), br. 4.13 (1H C-30 (H) (5.34 (1H, C-12)H)).

Mass: m/e 486 (M^+, 37%) 468 (1.47%), 454 (100.00%), 436 (6.61%), 424 (7.35%), 379 (4.28%) 357(80.88%), 278 (Fragment a, 5.88%), 260 (7.35%), 246 (44.11%) 234 (31.61%), 216 (80.80%), 207 (Fragment b, 59.55%), 189 (42.64%).

PRIDENTIGENIN D

Pridentigenin D was isolated from the middle fractions eluted with $CHCl_3$ and $CHCl_3$: MeOH (90:10 v/v) along with pridentigenin C and very small amount of pridentigenin E. It was isolated in pure form through preprative thick layer chromatography on silica gel G, with $CHCl_3$:MeOH (9:1 v/v) as a solvent system. Pridentigenin D was crystallized from Et_2O into small white crystals m.p. 170°C.

IR(KBr): 3360 cm^{-1} br(OH), 1700 cm^{-1} (carbonyl group) 1612 cm^{-1} (C=C stratching).

NMR($CDCl_3$): (400 MHz) δ 0.78 (3H, s, 24-Me), 0.88 (3H, s, 26-Me), 0.94 (3H, s, 25-Me), 0.97 (3H, s, 29-Me), 1.05 (3H, s, 23-Me), 1.23 (3H, s, 27-Me), 3.19 (ddJ = 11.5 Hz, 5.5 Hz, H-3a), 2.14 and 2.71 (d each, H = 16Hz, 2 x H-15), 3.43 and 3.87 (d each J=8 Hz, 2 x H-28), 3.32 and 3.58 (d each J=11 Hz, 2 x H-30).

Mass: 472(M^+), 455 (24.05%), 454 (61.32%), 441 (12.50%), 442 (12%), 423 (8.49%), 411 (10.37%), 357 (347%), 264 (Fragment a, 100.00%), 251 76%), 246 (23.58%), 233 (31.60%), 219(57%), 215(57.07%), 207 (Fragment b, 30.66/), 203 67.45%), 189 (153.30%). High resolution MS. 472.35097, calc. for $C_{30}H_{48}O_4$ 472.35520.

PRIDENTIGENIN D DIACETATE

Pridentigenin D (15 mg) was dissolved in ½ ml of Ac_2O to which one drop of pyridine was added, the reaction mixture was kept overnight at room temperature. and was worked up by adding ice, which furnished white precipitate which was filtered and dried. Crystallization of the acetyl derivative could not be achieved.

IR ($CHCl_3$): 1715 cm^{-1} (OAc), 1700 cm^{-1} (carbonyl), 1600 cm^{-1} *(C=C).*

Mass: m/e 556(6.95%) M^+, 496 (185%), 454 (5.4%), 412 (5.0%) 306 (61.3 %), 293 (49.0%), 275 (20 %), 261 (31%), 229 (5 %), 215 (26.2%), 203 (63.3%), 175 (25%), 147(35.1%).

PRIDENTIGENIN E

The fractions eluted with $CHCl_3$:MeOH (8:2 v/v) and pure methanol contained another compound along with some polar and some less polar impurities. Pure pridentigenin E was isolated by thick layer preprative chromatography of these fractions using $CHCl_3$:MeOH (8:2 v/v) as developing system and the band was made visible by spraying with water. The bands were scratched and extracted with MeOH, filtered, concentrated whereby the colourless crystals of pridentigenin E were obtained m.p., 268-270. On recrystallization from MeOH the melting point raised to 268-270°.

UV (methanol): λ_{max} 212 nm

IR (KBr): 3340 cm^{-1} br (OH), other peaks at 163: (C=C), 2950, 2870, 1382, 1378, 1100, 1042, 1025, 1000, 920 and 824 cm^{-1}.

NMR (C_6D_5N): δ 1.08 (6H, 2 x CH_3), 1.25 (3H, 1 x CH_3), 1.37 (3H, 1 x CH_3), 1.84 (6H, 2 x CH_3), 3.71 (C-28 (H_2–OH)), 3.97 C-30(H_2–OH), 4.64 (br 1 H, C-12).

Mass: m/e 474 (M^+, 6.60%), 456 (17.92%), 443 (10.37%), 438 (4.24%), 425 (15.09%), 407 (3.30%), 266 (Fragment a, 27.35%), 248 (14.15%), 236 (100.00%), 235 (81.35%), 217 (36.79%), 207 (Fragment b, 30.18%), 289 (17.45%).

PRIDENTIGENIN E TETRAACETATE

Pridentigenin E (50 mg) was dissolved in minimum quantity of Ac_2O, to which one drop of pyridine was added and kept at room temperature. Ice was added to the reaction mixture resulting in white precipitates. The precipitate were filtered, and dried, m.p. 186°. The purity of the acetylated product was checked on t.l.c. It could not be obtained in crystallised form.

IR($CHCl_3$): 1720 and 1710 cm^{-1} (OAc) 1500, 1410, 1390, 1190, 1020, 980, 960, and 920 cm^{-1}.

NMR($CDCl_3$): δ 0.81 (6H, 2 x CH_3), 0.9 (3H, 1 x CH_3), 0.93 (6H, 2 x CH_3), 1.25 (3H, 1 x CH_3), 2.07 (3H, OAc), 2.04 (3H, OAc), 2.06 (3H, OAc), 2.07 (3H, OAc), 3.87 (a4 H J_{AB}=7.2Hz), 4.48 t. (t1 H, C-3), 5.10 (m 1H, C-12) 5.35 (g 1H, C-16).

Mass: m/e 584(M^+–CH_3COOH, 10.90%), 522 (26.36%), 462 (5.45%), 449 (10.90%), 392 (Fragment a, 2.27%), 332 (23.63%), 272 (47.27%), 249 (Fragment b, 12.7%), 212 (25%), 199 (100%), 189(47.72%).

ACETONIDE OF PRIDENTIGENIN E

Acetonide of pridentigenin E was prepared by the method already described in the case of pridentigenin B. Starting material was not completely converted into acetonide. The less polar acetonide was separated by preparative thick layer chromatography using $CHCl_3$: MeOH (9:1 v/v) as developing solvent.

IR $CHCl_3$) 3400 (br, OH), 1600 (C=C).

Mass: m/e 514(M^+, 9.47%), 499 (6.84%) 456 (12.10%), 441 (21.05%), 439 (14.27%), 426 (21.05%), 42 (16.31%), 395(11.57%), 306(Fragment a, 100.00%) 291 (34.73%), 248 (45.28%), 231 (58.42%), 217 (47.36%). 207 (Fragment b, 65.78%), 189 (42.10%).

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