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BIOMIMETIC TRANSFORMATION OF A GUAIANOLIDE TO A PSEUDOGUAIANOLIDE

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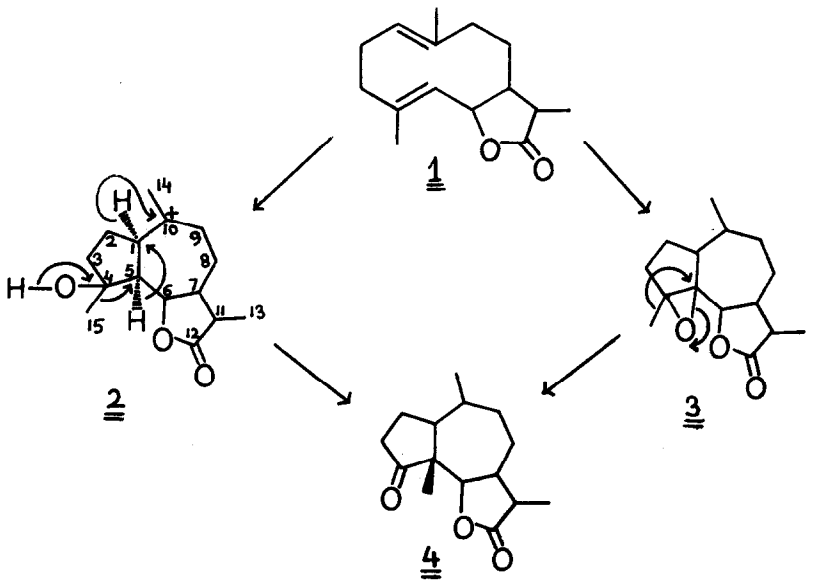
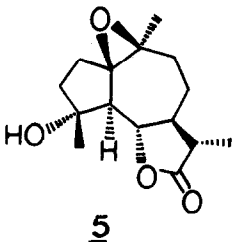
Abstract - The long awaited transformation of the guaianolide skeleton to the pseudoguaianolide skeleton (10 ⟶ 34) has been achieved. During this study two new carbon skeletons in sesquiterpene lactones have been reported.

Pseudoguaianolides are a class of sesquiterpene lactones formed *in vivo* by rearrangement of the guaianolide skeleton (2 or 3) which in turn is formed from the germacranolide skeleton 1 the immediate successor of farnesyl pyrophosphate. Historically, the discovery of pseudoguaianolides was made by Herz *et al* long time ago.[1] Since most of the naturally occurring pseudoguaianolides reported thus far, are found to be oxygenated at C-4, it was postulated by Herz that the pseudoguaianolide skeleton 4 is formed from the guaianolide skeleton 2 bearing a hydroxyl group at C-4 as shown in scheme I[2,3]. This postulation was made taking into

SCHEME I



consideration the stereochemistry at C-1 and C-10 in some of the naturally occurring pseudoguaianolides. In 1984 Bohlmann *et al* suggested that the pseudoguaianolide skeleton **4** is formed from the guaianolide skeleton **3** through rearrangement of the 4,5-epoxide function.[4] However, no biogenetic type *in vitro* conversion of the guaianolide skeleton to the pseudoguaianolide skeleton - a process which must involve a C-4 to C-5 methyl shift has yet been reported. It may be mentioned here that *in vitro* formation of the guaianolide skeleton from its biogenetic precursor the germacranolide, has been reported several times[5-7]. The only attempt for the conversion of a guaianolide to a pseudoguaianolide has been made by Fischer *et al*[8] who treated the epoxide **5** with BF_3.etherate with the hope of obtaining the corresponding pseudoguaianolide skeleton **6**. However, two major products obtained from this reaction were characterized as **7** and **8**.



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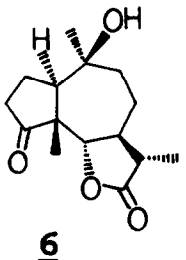

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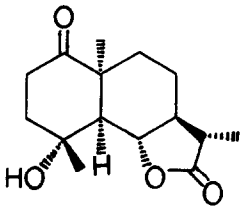

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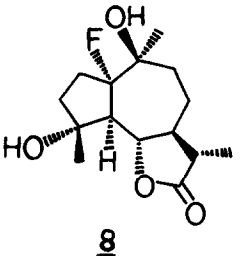

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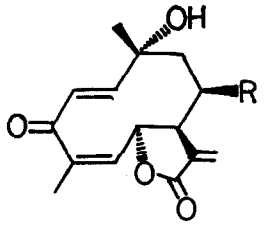
Due to availability of a large amount of tagitinin C **9** from *Tithonia diversifolia* (Hemsl.) A Gray[9] experiments were designed to use this compound as a starting material for a possible biogenetic type transformation of a guaianolide to a pseudoguaianolide[10]. Reaction of tagitinin C **9** with $SnCl_2$ furnishes cyclotagitinin C **10**[11]. Sodium borohydride reduction of cyclotagitinin C **10** furnished the tetrahydrocompound **11**, which on exposure to m-chloroperbenzoic acid (MCPBA) furnished a mixture of three compounds which were separated by preparative TLC (EtOAc:Pet ether , 4:1). The most polar compound obtained as a gum was identified as the epoxide **12** (for stereochemistry see later) and the least polar compound, m.p. 218°C, was identified as **13** on the basis of its spectral data.

The third compound was obtained as a solid, m.p. 146°C, whose ^1H NMR spectrum displayed two doublets with J=7 Hz each, at 1.20 and 1.28 ppm, the former integrating to six protons and the latter to three protons. Two singlets each integrating to three protons appeared at 1.38 and 1.62 ppm, a double doublet with J= 1& 7 Hz appeared at 4.27 ppm which was assigned to the proton under the hydroxyl,



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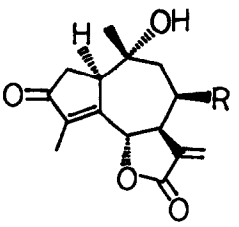

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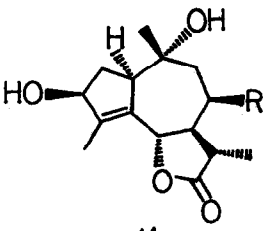

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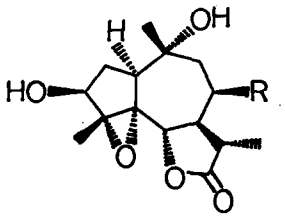

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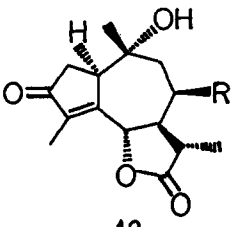

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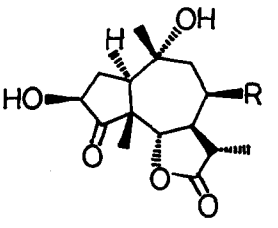

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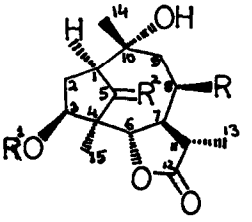


R=OCOCHMe$_2$

15 R^1=H ; R^2=O

16 R^1=Ac ; R^2=O

17 R^1=Ac ; R^2=H, α-OH

18 R^1=Ac ; R^2=H, α-OAc

a doublet with J=11 Hz at 4.92 ppm was assigned to H-6 and a multiplet at 5.40 ppm was assigned to H-8. The appearance of two doublets of doublets of doublets with J=7, 10 and 17 Hz and 1, 2.5 and 17 Hz, at 2.20 and 2.05 ppm respectively each integrating to one proton suggested that some change in the carbon skeleton has taken place. Since the IR spectrum displayed strong absorption bands at 1775, 1750 and 1740 cm^{-1}; this in conjunction with the ^1H NMR data led us to conceive structure 14 and 15 for this compound. As this compound was unreactive to sodium metaperiodate oxidation, the possibility of structure 14 could be easily ruled out and therefore, structure 15 was assigned to this compound.

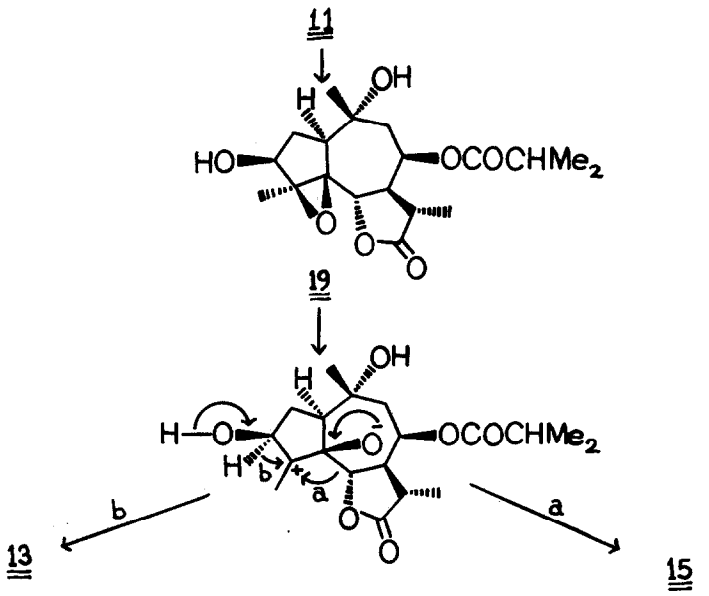
Structure 15 was further confirmed by converting it into the acetate 16 which on reduction with sodium borohydride furnished compound 17. Compound 17 was found to be unstable, however its acetate 18 obtained as a gum by overnight treatment with acetic anhydride and pyridine was found to be quite stable. Structure of 18 was established by its ^1H-^1H 2D COSY NMR spectrum[12-14] wherein long range coupling between H-1 and H-14, H-9a and H-14, H-3 and H-15 was observed. H-5 was long range coupled to H-2a (β) but no coupling between H-1 and H-5 was observed. This is in agreement with the molecular model of 18 in which the dihedral angle between H-1 and H-5β is 90° and there exists a w-relationship between H-5β and H-2a (β).

When the ^1H NMR spectrum of compound 15 was recorded in presence of trichloroacetylisocynate (TAI), the C-10 methyl and the proton at C-3 underwent para-

magnetic shift thus confirming the formation of dicarbamate ester derivative. H-1 underwent paramagnetic shift from 2.58 ppm to 3.56 ppm indicating cis-relationship between H-1 and C-10 hydroxyl group. Since the methyl at C-4 did not undergo any significant shift to the lower field region, it was assigned α-configuration and this conclusion was reinforced by NOE difference spectra of 18.

When the epoxide 12 was left overnight with MCPBA, no reaction took place and the starting material was recovered unchanged quantitatively. One would therefore conclude that the products 13 and 15 are formed from the β-epoxide 19 which might be unstable under the reaction conditions. Scheme II rationalizes the formation of compounds 13 and 15.

SCHEME II

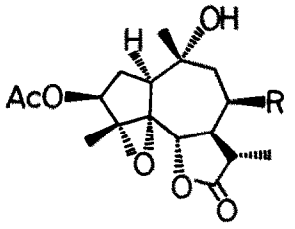


Acetylation of compound 12 furnished the acetate 20 which on exposure to $BF_3.Et_2O$ furnished a mixture of seven compounds. These were separated by preparative TLC (EtOAc:Pet ether, 1:1, three developments) and are designated as A-G for the purpose of discussion that follows.

Compound A was found to be unstable, gradually converting to a more polar compound identical with compound B. Therefore, on mild base treatment (5% $NaHCO_3$ in EtOH) it was quantitatively transformed into compound B, m.p. 152°C and whose spectral data established its identity as 22. Thus compound A was assigned structure 21.

Compound B analysed for $C_{17}H_{26}O_6$ and the IR spectrum showed absorption peaks at 1780, 1740 and 1680 cm^{-1} assignable to the lactone moiety, acetate and α,β-unsaturated ketone respectively. In the ^1H NMR spectrum, a two proton singlet at 6.08 ppm was assigned to H-8 and H-9, H-3 appeared as a double doublet with J=1.8 and 8.5 Hz at 4.85 ppm, H-6 as a triplet with J=10.2 Hz at 4.66 ppm, a doublet with J=7 Hz at 1.30 ppm was assigned to H-13, two sharp singlets at 1.50 and 1.58 ppm each integrating to three protons were assignable to H-15 and H-14 respectively, H-5 was clearly visible as a doublet with J=10.2 Hz at 1.90 ppm and H-2a and H-2b appeared as double doublets at 1.68 and 2.64 ppm with geminal coupling as 15 Hz and each coupled to H-3 with J=1.8 and 8.5 Hz respectively. When the ^1H NMR spectrum of 22 was recorded in presence of TAI, H-3 underwent paramagnetic shift from 4.85 ppm to 5.40 ppm. This experiment led to the conclusion that the hydroxyl at C-4 is

α-oriented and therefore the stereochemistry of the epoxide 12 is unequivocally fixed as α. The stereochemistry of the methyl group at C-1 as β was borne out from the mechanistic considerations.



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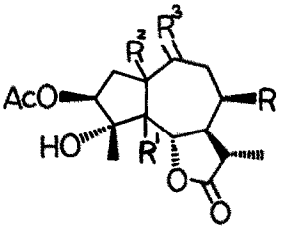
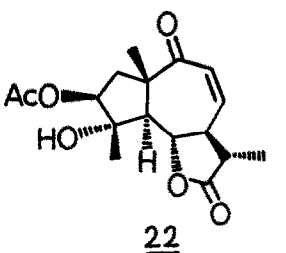
R=OCOCHMe$_2$



21 R^1=α-H; R^2=β-Me; R^3=O

23 R^1=β-F; R^2=α-H; R^3=β-Me, α-OH

24 R^1=α-H; R^2=β-F; R^3=β-Me, α-OH



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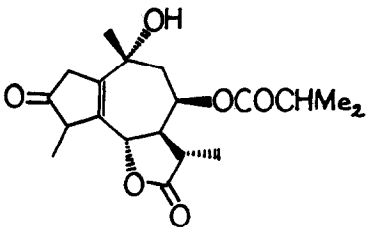
Compound C obtained as a gum revealed the presence of a fluorine atom at C-5 in the ^{1}H NMR spectrum as H-6 now appeared as a double doublet with J=10 and 25 Hz at 4.98 ppm. Such a high coupling of H-6 is consistent with the presence of a fluorine at C-5. Due to the presence of fluorine at C-5, H-15 appeared as a doublet with J=3 Hz at 1.45 ppm. The appearance of H-1 as a broad double doublet with J=10.5 and 34 Hz at 2.39 ppm left no doubt that fluorine is present at C-5. On the basis of above data structure 23 was assigned to compound C which was fully corroborated by its mass spectrum. High resolution mass spectrum although did not show the molecular ion peak but the mass measurement at m/z 370 (M^+-AcOH) led to the formula $C_{19}H_{27}O_6F$ for this ion. The β-stereochemistry to fluorine at C-5 was assigned on the basis of its coupling constants with H-6 and H-1 which agree with the dihedral angles as indicated by the molecular model of compound 23[15].

Compound D obtained as a gum gave the molecular ion peak at m/z 430 in the low resolution mass spectrum, thus indicating the presence of a fluorine atom in it. The ^{1}H NMR spectrum confirmed the position of fluorine at C-1 as H-14 now appeared as a doublet with J=2.4 Hz at 1.40 ppm and therefore structure 24 was assigned to compound D. Irradiation at H-6 located the presence of H-5 at 2.54 ppm as a doublet of doublets with J=10.5 & 36 Hz, thus suggesting the same stereochemical relationship between fluorine and hydrogen at C-5 and C-1 and vice-versa in compounds 23 and 24 respectively. Therefore, β-configuration was assigned to fluorine in compound 24[15].

Compound E obtained as a solid, m.p. 218°C, was characterized as 13 on the basis of its identical m.p., mixed m.p. and spectral data (IR, MS & NMR) with that of authentic 13.

Compound F, m.p. 178°C gave the molecular ion peak at m/z 350 in the low resolution mass spectrum. In the ^{1}H NMR spectrum H-8 appeared as a doublet of triplets with J=10 and 5 Hz at 5.49 ppm indicating a drastic change in the conformation of the seven membered ring and H-6 appeared as a doublet with J=11 Hz at 4.65 ppm suggesting the absence of hydrogen at C-5. Besides, C-15 methyl appeared as a doublet with J=7 Hz at 1.25 ppm. Therefore, structure 25 was assigned to compound F.

Compound F on stirring with silica gel in dichloromethane and methanol for 48 hours resulted in the formation of another crystalline compound, m.p. 190°, which was found to be identical with compound G. The identity of compound G was established on the basis of its spectral characteristics as 26. Since the m.p. and spectral data of compound 26 were different from that of 13, H-1 was assigned



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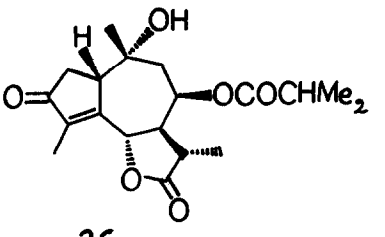

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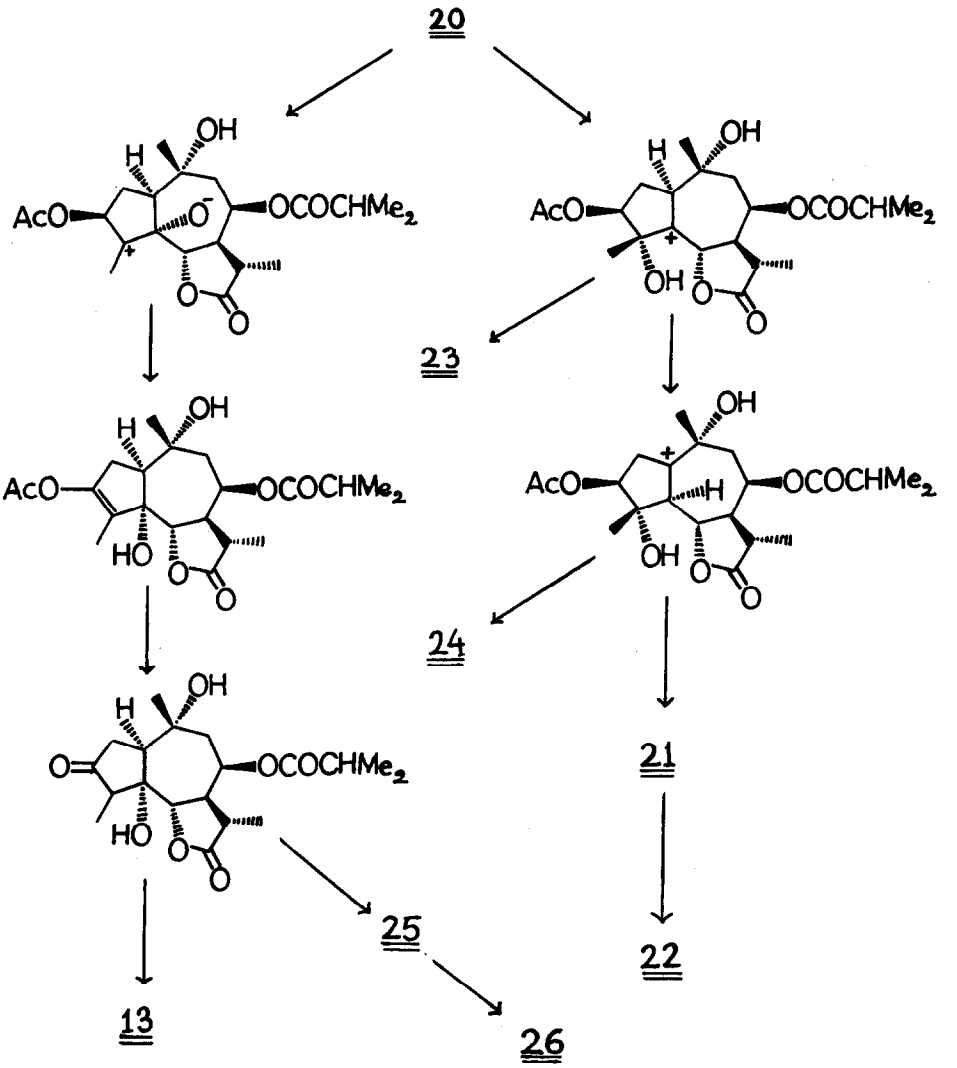
/3-stereochemistry. Besides, in compound 13, H-15 is coupled to H-1 ($J_{15,1}$=1.5 Hz) whereas in compound 26, this coupling is less than 1 Hz[16].

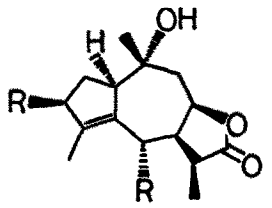
Formation of compounds 13, 21, 23-26 can be rationalized as shown in Scheme III. It was argued that the carbonium ion formed at C-5 is stabilized from the

SCHEME III

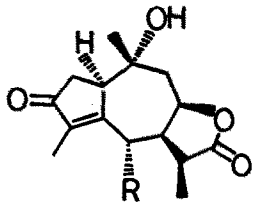


/3-side either by C-8 ester side chain or C-3 acetate or the solvent, therefore, the migration of H-1 occurs from the α-side generating a carbonium ion at C-1 and then the C-10 methyl migration leads to the formation of compound 21.

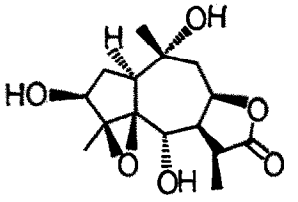
Having failed to get any product with the pseudoguaianolide skeleton, it was decided to carry out the rearrangement studies on the epoxide of 27, because most of the naturally occurring pseudoguaianolides contain lactone ring closed towards C-8.



27 R=OH
28 R=OAc



29 R=OH
30 R=OAc



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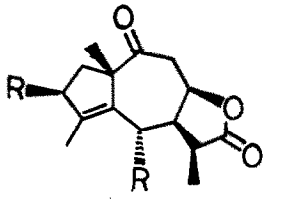


32 R=OH
33 R=OAc

Reaction of compound 11 with sodium methoxide in methanol furnished compound 27, m.p. 130°C. In the IR spectrum the only band present in the carbonyl region at 1770 cm^{-1} was due to the presence of the lactone moiety. The low resolution mass spectrum recorded the molecular ion peak at m/z 282 which was also confirmed by chemical ionization mass spectrum. The ^1H NMR spectrum was devoid of signals due to the ester side chain, H-8 appeared as a doublet of doublets of doublets with J=5, 7 and 11.5 Hz at 5.02 ppm, H-3 as a broad triplet with J=7 Hz at 4.39 ppm, H-14 as a singlet at 1.19 ppm, H-13 as a doublet with J=6.5 Hz at 1.25 ppm and H-15 as a multiplet at 1.69 ppm. The most significant change was observed for the proton at C-6 which appeared as a doublet with $J_{6,7}$=4.5 Hz at 4.64 ppm and this can be explained by changing the conformation of the seven membered ring [vide infra].

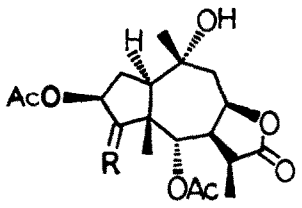
Treatment of compound 27 with MCPBA furnished one major epoxide 31 in whose ^1H NMR spectrum recorded in the presence of TAI, H-15 did not undergo any paramagnetic shift thus suggesting a trans-relationship between the hydroxyl at C-3 and the C-4 methyl. Therefore, /3-stereochemistry was assigned to the epoxide 31. Since H-7 underwent paramagnetic shift from 2.64 ppm to 3.2 ppm, it suggested a cis-relationship between the C-6 hydroxyl and H-7.[17]

The epoxide 31 on exposure to BF_3.etherate furnished a mixture of two products separated by preparative TLC (EtOAc:Pet.ether, 4:1). The less polar product was identified as 29 on the basis of its spectral data and decoupling experiments . Acetylation of compound 29 gave compound 30. The more polar compound was found to be unstable and was therefore acetylated. The diacetate was characterized as 33 on the basis of its spectral data and therefore structure 32 was

assigned to the more polar product.

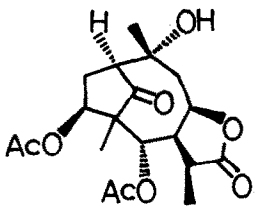
Acetylation of compound 27 furnished the diacetate 28, m.p. 125°C which on exposure to MCPBA furnished a mixture of several products from which four major products arbitrarily named as A-D were isolated.

Compound A obtained as a solid m.p. 135°C gave the molecular ion peak at m/z 382 in the low resolution mass spectrum. In the ^{1}H NMR spectrum, H-6 appeared as a doublet with J=5.5 Hz at 5.32 ppm, H-8 as a doublet of triplets with J=4.5 and 9 Hz at 5.08 ppm, H-3 appeared as a double doublet with J=1.5 and 6.5 Hz at 5.14 ppm, two singlets each integrating to three protons at 1.22 and 1.44 ppm were assigned to H-14 and H-15 respectively and H-13 appeared as a doublet with J=7 Hz at 1.34 ppm. Comparison of the above NMR data with that of compound 28 indicated a significant change in the chemical shift of H-3, H-6 and H-15 and therefore, structure 34 was assigned to compound A which was fully supported by its high resolution mass spectrum in which it analysed for $C_{19}H_{27}O_8$ [$(M+H)^+$ in the positive FAB mass spectrum]. ^{1}H NMR signals of compound 34 were assigned unequivocally by its COSY 45 spectrum[18]



34 R=O

35 R=H, β-OH



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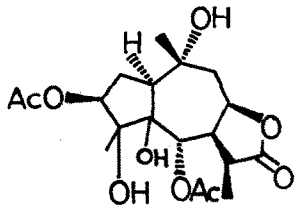

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Sodium borohydride reduction of compound 34 furnished the dihydro compound 35 in whose ^{1}H NMR spectrum H-3 was found to be coupled to H-4. ^{1}H NMR spectrum of compound 35 recorded in the presence of TAI resulted in a paramagnetic shift of H-15 and therefore β-configuration was assigned to the methyl at C-5.

Compound B was identified as 30 on the basis of its comparison (TLC, IR, NMR and MS) with the authentic sample.

Among the various possible rearrangement products containing the keto group which could be conceived from the transient epoxide of compound 28, structure 36 was considered to be the most likely for compound C on the basis of its spectral data especially the chemical shift of H-14 and H-15 in the NMR spectrum (compare with 15). Structure 36 was confirmed by its 2D COSY 45 spectrum.

The low resolution mass spectrum of compound D gave the highest peak at m/z 399 thus indicating that elements of water has been added to the transient epoxide of compound 28 and therefore structure 37 was assigned to it which was fully supported by its IR and NMR spectra.

EXPERIMENTAL

Melting points were determined on Buchi oil heating type melting point apparatus and are uncorrected. IR spectra were recorded as thin film in $CHCl_3$ on Perkin Elmer 237B spectrometer. The NMR spectra were recorded at 400 MHz (Brüker WH or AM) in $CDCl_3$ unless otherwise stated with TMS as internal standard. Chemical shifts are expressed as δ in ppm. Decoupling difference spectra (DDS) were recorded at 400 MHz. Mass spectra were recorded under electron impact at 70 ev on MS 30 spectrometer unless otherwise stated. Rotations were recorded on Jasco DIP-180. For preparative TLC silica gel G (E. Merck, India) was used. MCPBA used was obtained from Fluka, AC (Chem). Petroleum ether refers to the fraction b.p.

60-80°C. Solvents were dried over anhydrous sodium sulphate.

Fast atom bombardment (FAB) mass spectrometry[19-21] was carried out using a VG Micromass ZAB-2F mass spectrometer, an instrument with reverse geometry and fitted with a high field magnet and coupled to a VG 11-250 data system. The samples were loaded in thioglycerol on to a stainless steel probe and bombarded with xenon atoms having 8 KeV energy. During the high resolution FAB-MS measurements a resolving power of 25000 (10% valley definition) was used.

The two dimensional correlated ^1H NMR experiment was performed on Brüker WH 400. The applied pulse sequence was $\pi/2 - t_1 - \pi/2 - (FID\ t_2)$. The spectral widths in F_1 and F_2 were $\pm$ 1072.961 Hz and 2145.923 Hz respectively; the number of data points in F_2 being 2048 and F_1 512. After one level of zero filling transformed points became 2048 in F_1. Unshifted sine bell function was used.

The two dimensional COSY 45 ^1H NMR experiments were performed on Brüker AM 300 operated at 300 MHz. The applied pulse sequence was $\pi/2 - t_1 - \pi/4 - (FID\ t_2)$[18]. The spectral widths in F_1 and F_2 were 825.083 Hz and 1650.165 Hz for compound 36 and 1550 Hz for compound 34 respectively. The number of data points in F_2 was 1024 and F_1 was 512. t_1 was incremented 174 times for compound 34 and 79 times for compound 36 and each step consisted of 32 transients. After zero filling transformed data points became 1024 in the F_2 domain and 512 in the F_1 domain for both the compounds. Shifted sine bell multiplication was used in both domains with the maximum occurring in the center of the FIDS.

^1H-^{13}C chemical shift correlated experiment[22] was performed on Brüker AM 300 operated at 300 MHz for ^1H resonance and at 75 MHz for ^{13}C resonance. Spectral widths of 841.151 and 7142.857 Hz were used for dimensions δ_1 and δ_2 respectively. The number of acquisition data points were 2048 in δ_2 and 256 in δ_1 dimensions respectively. 800 transients were used for each of the 256 increments recorded.

ISOLATION OF TAGITININ C 9 AND ITS CYCLIZATION TO 10 :

Tagitinin C 9 was isolated[9] from Tithonia diversifolia (Hemsl.) A Grey collected from Lahoal area, Dibrugarh District, Assam, India and was cyclized to cyclotagitinin C 10 as described previously[10]

$NaBH_4$ REDUCTION OF 10 :

A solution of 240 mg of 10 in 5 ml methanol was cooled to 0°C and treated with 250 mg of sodium borohydride. The reaction mixture was stirred at 0°C for 10 min; diluted with water, acidified with dilute acetic acid and extracted with dichloromethane (6x75 ml). The washed and dried extract was evaporated and the residue was purified by preparative TLC (EtOAc:Pet ether, 4:1) to furnish 158 mg of 11 as a gum. IR (cm^{-1}): 3550, 1775 and 1730; ^1H NMR (270 MHz): 1.16 s (H-14), 1.20 d (J=7 Hz, H-3' & H-4'), 1.25 d (J=6.5 Hz, H-13), 1.65 m (H-2b), 1.87 dd br (J=15 & 4 Hz, H-9b), 1.93 m (H-15), 2.29 (H-9a), 2.30 (H-7, 11), 2.51 dt (J=15, 7.5 Hz, H-2a), 2.59 sept (J=7 Hz, H-2'), 3.00 m (H-1), 4.56 d (J=7.5 Hz, H-3), 5.15 d br (J=9 Hz, H-6), 5.37 t br (J=4 Hz, H-8). MS:m/z at 352 (M^+), 282, 264, 246, 228 and 71.

REACTION OF 11 WITH MCPBA :

To a solution of 150 mg of 11 in 3 ml of dry dichloromethane was added 300 mg of MCPBA and the reaction mixture was stirred at room temperature monitoring by TLC. After 4 hours when the reaction was complete, it was diluted with 300 ml of dichloromethane, washed successively with dilute solutions of potassium iodide, sodium thiosulphate and sodium bicarbonate and finally with water. The washed and dried extract was then evaporated and the residue showed three spots on TLC which were isolated by preparative TLC (EtOAc:Pet ether, 4:1) to give 98 mg of the more polar 12 as a gum, 15 mg of the least polar 13 (m.p. 218°C, hexane-EtOAc) and 23 mg of compound 15 (m.p. 146°C, hexane-EtOAc). Spectral data of 12 : IR (cm^{-1}): 3500, 1780, 1740, 1460, 1400, 1325, 1250, 1175, 1150 and 1075; ^1H NMR: 1.20 d, 1.23 d (J=7 Hz, H-3' & H-4'), 1.27 d (J=7 Hz, H-13), 1.40 s (H-14), 1.63 s (H-15), 1.45 dd (J=4, 15 Hz, H-9a), 2.10 dd (J=4 & 15 Hz, H-9b), 2.20-2.60 (overlapping signals of H-1, H-2a, H-2b, H-7 & H-2'), 3.95 t br (J=8 Hz, H-3), 4.95 d (J=10.5 Hz, H-6), 5.40 t (J=4 Hz, H-8). MS:m/z at 368 (M^+), 352, 338, 334, 321 and 263.

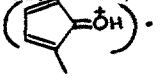
Spectral data of 13 : IR (cm^{-1}): 3500, 2950, 1775, 1740, 1700, 1460, 1390, 1315, 1250, 1225, 1175, 1140, 1115, 1060 and 995; ^1H NMR (Assignment by ^1H-^1H COSY 2D NMR): 1.09 s br (H-14), 1.23 d (J=7 Hz, H-3'), 1.24 d (J=7 Hz, H-4'), 1.33 d (J=7 Hz, H-13), 1.95 t (J=1.5 Hz, H-15), 2.03 dd br (J=15 & 3.5 Hz, H-9a), 2.40 (H-11), 2.40 (H-9b), 2.45 (H-7), 2.60 (H-2a), 2.60 (H-2b), 2.62 sept (J=7 Hz, H-2'), 3.31 m (H-1), 5.29 d br (J=10 Hz, H-6) and 5.44 t br (J=3.5 Hz, H-8). MS:m/z at 350 (M^+), 280, 262 and 71. Calc. for $C_{19}H_{26}O_6$: mol. wt. 350.1727 and found mol.wt. (MS) 350.1729.

Spectral data of 15 : IR (cm^{-1}) : 3500, 2900, 1775, 1750, 1740, 1460, 1390, 1325, 1260, 1240, 1175, 1150, 1115, 1050 and 1015; ^1H NMR: 1.20 d (J=7 Hz, H-3' & H-4'), 1.28 d (J=7 Hz, H-13), 1.38 s (H-14), 1.62 s (H-15), 1.93 dd (J=4 & 15 Hz, H-9a), 2.05 ddd (J=1, 2.5 & 17 Hz, H-2a), 2.20 ddd (J=7, 10 & 17 Hz, H-2b), 2.35 dd (J=1 & 15 Hz, H-9b), 2.36 m (H-11), 2.52 ddd (J=1.5, 11 & 12 Hz, H-7), 2.58 dd (J=2.5 & 10 Hz, H-1), 2.65 sept (J=7 Hz, H-2'), 4.27 dd (J=1 & 7 Hz, H-3), 4.92 d (J=11 Hz,

H-6) and 5.40 m (H-8); ^{13}C NMR: 10.90 q (C-13), 12.92 q (C-15), 19.02 q (C-3' & C-4'), 24.94 q (C-14), 34.34 t (C-2), 36.78 d (C-7), 49.60 d (C-11), 49.84 d (C-2'), 53.44 t (C-9), 66.01 d (C-1), 69.37 d (C-8), 70.40 (C-4 or C-10), 71.56 (C-4 or C-10), 71.99 d (C-6), 74.31 d (C-3), 175.87, 176.36 and 176.36 (C-5, C-12 and C-1') MS: m/z at 368 (M^+), 350, 298, 280, 262 and 261.

^{1}H NMR of (15 + TAI) : 1.20 d (J=7 Hz, H-3' & H-4'), 1.32 d (J=7 Hz, H-13), 1.60 s (H-14), 1.64 s (H-15), 2.00 dd (J=15 & 4 Hz, H-9a), 2.60 ddd (J=1.5, 11 & 12 Hz, H-7), 2.68 sept (J=7 Hz, H-2'), 2.72 dd (J=1 & 15 Hz, H-9b), 3.56 dd (J=2.5 & 10 Hz H-1), 4.86 d (J=11 Hz, H-6), 5.36 dd (J=1 & 7 Hz, H-3), 5.48 m (H-8), 8.24 s br and 8.44 s br (two NH).

ACETYLATION OF 15 :

To a solution of 10 mg of 15 in 1 ml of pyridine was added 1 ml of acetic anhydride and the reaction mixture was left overnight at room temperature. The reaction mixture was then diluted with 200 ml of water and extracted with dichloromethane (3x75 ml). The washed and dried extract was evaporated and pyridine was removed by co-distillating with toluene in vacuo. The residue obtained was purified by preparative TLC (EtOAc:Hexane, 1:1) to have 10 mg of the acetate 16 as a gum. IR (cm^{-1}): 3450, 2950, 2925, 1775, 1750, 1740, 1460, 1390, 1325, 1255, 1175, 1150, 1050 and 1025; ^{1}H NMR (Assignment by DDS): 1.22 d (J=7 Hz, H-3'), 1.23 d (J=7 Hz, H-4'), 1.31 s (H-14), 1.52 s (H-15), 1.29 d (J=7 Hz, H-13), 2.11 s (acetate), 1.90 dd br (J=15 & 4 Hz, H-9a), 2.05 ddd (J=1, 2.5 & 17 Hz, H-2a), 2.20 ddd (J=7, 10 & 17 Hz, H-2b), 2.35 dd (J=4 & 15 Hz, H-9b), 2.35 dq (J=6.5 & 12 Hz, H-11), 2.59 dd (J=10 & 2.5 Hz, H-1), 2.52 ddd (J=1.5, 11 & 12 Hz, H-7), 2.64 sept (J=7 Hz, H-2'), 4.85 d (J=11 Hz, H-6), 5.23 dd (J=1 & 7 Hz, H-3) and 5.38 dt (J=1.5 & 4 Hz, H-8). MS: m/z at 410 (M^+), 367 (M^+-Ac), 350 (M^+-Ac-OH), 332 (M^+-Ac-OH-H_2O), 322 (M^+-$C_4H_8O_2$) 304 (M^+-$C_4H_8O_2$- H_2O), 280, 262 (Base peak), 247, 237, 219, 207, 189, 175, 167, 156, 139, 124, 111, 95 and 71. (+) FAB MS: m/z at 433.2 [(M+Na)$^+$], 412.2, 411.2 [(M+H)$^+$] 393.2, 351.2, 334.2, 333.2, 323.1, 305.1, 281, 263.1, 245.1, 235.1, 217.1, 203.1, 189.1, 175.1, 161.1, 147.1, 133.1, 123.1, 111.1 and 95.1  .

Measured mass of the $(M+H)^+$ ion is 411.1995 (FAB MS), mass calculated for $C_{21}H_{31}O_8$ is 411.2018.

$NaIO_4$ TREATMENT OF 15 :

To a solution of 5 mg of 15 in 1 ml of methanol was added four drops of the saturated solution of $NaIO_4$ in water and left in the dark. After 72 hours the reaction mixture was diluted with 100 ml of dichloromethane and washed with water. Evaporation of the solvent furnished the starting material back in a quantitative yield.

CONVERSION OF 16 INTO 18 :

To a solution of 20 mg of 16 in 2 ml of methanol at 0°C was added 40 mg of sodium borohydride and stirred at 0°C. After 10 min the reaction mixture was diluted with 100 ml of water, acidified with dilute acetic acid and extracted with dichloromethane (5x75 ml). The washed and dried extract was evaporated and the residue thus obtained was purified on TLC to furnish 20 mg of 17 as a gum. Compound 17 was found to be unstable and therefore, no satisfactory spectral and analytical data could be obtained. However, its diacetate 18 (17 mg) obtained as a gum by overnight treatment with acetic anhydride-pyridine and after usual work up followed by purification by preparative TLC (EtOAc:Hexane, 1:1) was quite stable. $[\alpha]_D^{27}$ = -12° (c 1% $CHCl_3$); CD_{MeOH} $\Delta\epsilon_{320}$ = +0.6 and $\Delta\epsilon_{340}$ = -0.3; IR (cm^{-1}): 3450, 3000, 2950, 1775-1725 (very strong and broad band), 1460, 1375, 1250, 1205, 1150, 1125, 1070, 1040 and 975 ; ^{1}H NMR (500 MHz) (Assignment by ^{1}H-^{1}H COSY 2D NMR): 1.02 d (J=6.7 Hz, H-13), 1.19 d (J=7 Hz, H-3'), 1.20 d (J=7 Hz, H-4'), 1.27 s br (H-14), 1.44 s (H-15), 1.84 ddd (J=0.7, 4.3 & 15.1 Hz, H-9a), 1.95.2.05 (overlapping signals of H-2a and H-11), 2.06 (acetate), 2.06 (acetate), 2.17 ddd (J=7.2, 10.0 & 17.0 Hz, H-2b), 2.27 dd (J=3.2 & 15.1 Hz, H-9b), 2.39 ddd (J=1.5, 10.0 and 11.9 Hz, H-7), 2.51 dd (J=3.1 & 10.0 Hz, H-1), 2.59 sept (J=7 Hz, H-2'), 4.66 d (J=10 Hz, H-6), 5.15 dd (J=1.4 & 7.2 Hz, H-3), 5.38 ddd (J=1.5, 3.2 & 4.3 Hz, H-8) and 6.05 d (J=4.5 Hz, H-5); ^{13}C NMR (75 MHz, assignment by ^{1}H-^{13}C 2D COSY and DEPT NMR experiments) : 10.51 q (C-13), 11.18 q (C-15), 19.06 q (C-3'), 19.12 q (C-4'), 21.17 q (acetate Me), 21.33 q (acetate Me), 24.42 q (C-14), 24.42 q (C-14), 32.42 t C-2), 34.34 d (C-2'), 39.32 d (C-11), 48.92 d (C-7), 50.44 t (C-9), 53.47 d (C-1), 65.70 d (C-8), 68.99 s (C-4 or C-10), 72.38 s (C-4 or C-10), 73.85 d (C-6), 76.41 d (C-3), 98.54 d (C-5), 170.00 (C-12), 170.57 (acetate C=O), 176.04 (C-1'). MS: m/z at 410 (M^+-Ac-H), 395, 394, 392, 375, 333, 332, 317, 316, 306, 305, 288, 256, 255, 254, 248, 247, 246, 203 and 71. CI MS (CH_4): m/z at 456 (M+2), 439, 438, 410, 398, 397, 396, 392, 382, 381, 379, 378, 377, 248 and 230.

TREATMENT OF 12 WITH MCPBA :

To a solution of 50 mg of 12 in 1 ml of dry dichloromethane was added 100 mg of m-chloroperbenzoic acid and the reaction mixture was left for overnight stirring at room temperature. It was diluted with 200 ml of dichloromethane, washed successively with dilute solutions of potassium iodide, sodium thiosulphate and sodium bicarbonate and finally with water. The dried solution was then evaporated to get a residue which was identical with 12 on TLC, IR, MS and NMR spectra.

ACETYLATION OF 12 :

To a solution of 95 mg of 12 in 3 ml dry pyridine was added 3 ml acetic anhydride and was left overnight at room temperature. The reaction mixture was worked up as usual and the residue purified by preparative TLC to furnish 90 mg of 20 as a gum (EtOAc:Hexane, 1:1). IR (cm^{-1}): 3500, 1780, 1740, 1460, 1400, 1325, 1250, 1175, 1150 and 1075; ^1H NMR (200 MHz): 1.21 d (J=7 Hz, H-3' & H-4'), 1.29 d (J=6.5 Hz, H-13), 1.39 s (H-14), 1.61 s (H-15), 1.90 dd br (J=15 & 4 Hz, H-9a), 2.12 s (acetate), 2.10-2.50 (overlapping signals of H-1, H-2, H-7, H-9b & H-11), 2.60 sept (J=7 Hz, H-2'), 4.90 d (J=10.5 Hz, H-6), 4.95 dt (J=1 & 8 Hz, H-3) and 5.40 t br (J=3 Hz, H-8). MS:m/z at 368 (M^+-CH_2CO), 351, 335, 323 and 318. Measured mass of the (M^+-CH_2CO) ion is 368.1822, mass calcd. 368.1835 for $C_{19}H_{28}O_7$.

REACTION OF 20 WITH $BF_3.Et_2O$:

To a solution of 90 mg of 20 in 0.5 ml of dry dioxane and 3 ml of dry diethyl ether chilled to 0°C was added 8 drops of freshly distilled (over CaH_2) $BF_3.Et_2O$. The reaction mixture was kept at room temperature for overnight, diluted with 200 ml of ether, washed with water, and dried solution was evaporated. The gummy residue gave six major spots on TLC. Separation by preparative TLC (EtOAc: Hexane, 1:2, three developments) furnished 13 (8 mg, m.p. 218°C, Hexane-EtOAc), 21 (25.2 mg as a gum), 23 (9.6 mg as a gum), 24 (8 mg as a gum), 25 (20 mg, m.p. 178°C, EtOAc) and 26 (11 mg, m.p. 190°C, EtOAc).

Compound 13 was characterized on the basis of its identical spectral data (IR, NMR & MS) with that of authentic 13. Mixed melting points with the authentic sample also showed no depression.

As 21 was found to be unstable, its satisfactory analytical data could not be obtained.

CONVERSION OF 21 INTO 22 :

To a solution of 15 mg of 21 in 1 ml ethanol was added a few drops of 5% solution of $NaHCO_3$ in water and left at room temperature overnight. The reaction mixture was diluted with 100 ml of dichloromethane, washed with water and dried. Evaporation of the dried solution and purification of the residual mass by preparative TLC (EtOAc:Pet.ether, 1:1) furnished 10 mg of 22 as a gum which crystallized from chloroform in three days, m.p. 152°C. IR (cm^{-1}): 3450, 1780, 1740, 1685, 1475, 1440, 1390, 1275, 1240, 1225, 1195, 1110, 1060 and 1000; ^1H NMR: 1.30 d (J=7 Hz, H-13), 1.50 s (C-15), 1.58 s (H-14), 1.68 dd (J=15 & 1.8 Hz, H-2a), 1.90 d (J=10.2 Hz, H-5), 2.10 s (acetate), 2.55 (overlapping signals of H-7 & H-11), 2.64 dd (J=15 & 8.5 Hz, H-2b), 4.66 t (J=10.2 Hz, H-6), 4.85 dd (J=1.8 & 8.5 Hz, H-3) and 6.08 s (two protons, H-8 & H-9). MS:m/z at 322 (M^+), 279, 263, 262, 246, 239, 237, 218 and 203. Measured mass of the M^+ ion is 322.1394 (EIMS), mass calcd. for $C_{17}H_{22}O_6$ is 322.1417. ^1H NMR of (22 + TAI) (60 MHz): 1.36 d (J=7 Hz, H-13), 1.58 s (H-15), 2.10 d (J=10.2 Hz, H-5), 5.00 t (J=10 Hz, H-6), 5.40 dd (J=1.8 & 8.5 Hz, H-3), 6.10 s (two protons, H-8 & H-9) and 8.30 s br (NH).

Spectral data of 23 : IR (cm^{-1}): 3500, 1775, 1750, 1685 and 900. ^1H NMR (Assignment by DDS): 1.20 d, 1.21 d (J=7 Hz, H-3' & H-4'), 1.27 d (J=7 Hz, H-13), 1.35 s (H-14) 1.45 d (J=3 Hz, H-15), 1.43 m (H-9a), 1.79 m (H-2a), 1.95 dd (J=5.5 & 15 Hz, H-9b), 2.00 s (acetate), 2.25 (overlapping signals of H-7 & H-2b), 2.39 dd br (J=10.5 & 34 Hz, H-1), 2.60 sept (J=6.5 Hz, H-2'), 4.85 ddd (J=3, 7 & 10 Hz, H-3), 4.98 dd (J=10 & 25 Hz, H-6) and 5.29 ddd (J=2.5, 4 & 5.5 Hz, H-8); MS:m/z at 370.1512 ($M^+-CH_3CO_2H$), 350, 342, 332, 330, 327, and 307. Mass calcd. for $C_{19}H_{27}O_6F$ ($M^+-CH_3CO_2H$) is 370.1519.

Spectral data of 24 : IR (cm^{-1}): 3500, 1775, 1750, 1680 and 900; ^1H NMR: 1.17 d, 1.19 d (J=7 Hz, H-3' & H-4'), 1.27 d (J=7 Hz, H-13), 1.33 s (H-15), 1.40 d (J=2.4 Hz, H-14), 2.13 s (acetate), 2.59 sept (J=7 Hz, H-2'), 4.97 t br (J=10.5 Hz, H-6), 5.00 d br (J=10.4 Hz, H-3) and 5.27 m (H-8). MS:m/z at 430 (M^+), 370, 368, 350, 342, 332, 330, 327 and 307.

Spectral data of 25 : IR (cm^{-1}): 3500, 1775, 1740, 1460, 1405, 1380, 1325, 1250, 1205, 1175 and 1150; ^1H NMR (Assignment by DDS): 1.20 d, 1.21 d (J=7 Hz, H-3' & H-4'), 1.23 d (J=7 Hz, H-13), 1.25 d (J=7 Hz, H-15), 1.40 s (H-14), 2.08 dd (J=5 & 15 Hz, H-9a), 2.20 dd (J=10 & 15 Hz, H-9b), 2.45q (J=7 Hz, H-4), 2.54 (2 protons, centre of AB system, H-2a & H-2b), 2.61 sept (J=7 Hz, H-2'), 2.60 (overlapping signals of H-7 & H-11), 4.65 d (J=11 Hz, H-6) and 5.49 dt (J=5 & 10 Hz, H-8). MS:m/z at 350 (M^+), 332, 280, 262 and 244.

Spectral data of 26 : IR (cm^{-1}): 3450, 2900, 1780, 1740, 1710, 1460, 1405, 1395, 1325, 1250, 1205, 1175, 1125, 1075, 1050 and 995; ^1H NMR (Assignment by DDS): 1.22 d, 1.23 d (J=7 Hz, H-3' and H-4'), 1.31 d (J=7 Hz, H-13), 1.44 s (H-14), 1.65 s br (H-15), 1.98 dd (=14 & 15 Hz, H-9a), 2.34 dd (J=5 & 15 Hz, H-9b), 2.28 dd (J=7.5 & 17 Hz, H-2a), 2.36 dq (J=7 & 11.5 Hz, H-11), 2.42 dd (J=8.5 & 17 Hz, H-2b), 2.50 ddd (J=1, 11 & 11.5 Hz, H-7), 2.64 sept (J=7 Hz, H-2'), 2.79 dd (J=7.5 & 8.5 Hz, H-1), 4.97 d (J=11 Hz, H-6) and 5.44 ddd (J=5, 4 & 1 Hz, H-8). MS:m/z at 350 (M^+), 280, 262 and 71.

CONVERSION OF 25 INTO 26 :

To a solution of 10 mg of 25 in 2 ml of dichloromethane and 1 ml of methanol was added 200 mg of silica gel G for TLC (E Merck, India), stirred for 48 hours.

Then the reaction mixture was filtered, silica gel was washed with 25 ml CH_2Cl_2 and the the solvent evaporated under reduced pressure to furnish a gummy mass. Preparative TLC (EtOAc:Pet.ether, 1:1) of the gum afforded crystalline 5 mg of 25 and crystalline 3 mg of 26.

CONVERSION OF 11 INTO 27 :

A solution of 100 mg of 11 in 2 ml of dry methanol was treated with 1 ml of sodium methoxide solution (0.5 M, obtained by dissolving ~25 mg of sodium metal in 1 ml of methanol at 0°C) in methanol at 0°C and the reaction mixture was kept overnight at room temperature. It was then diluted with cold water, acidified with dilute acetic acid and extracted with ethyl acetate (5x75 ml). The dried extract was evaporated under reduced pressure, acetic acid was removed by co-distillating with toluene under vaccum. Purification by preparative TLC (EtOAc:Hexane, 4:1), furnished 70 mg of 27 as the major product m.p. 130°C (EtOAc). IR (cm^{-1}) : 3500, 1770, 1450, 1390, 1305, 1190, 1150, 1060 and 975; ^1H NMR (270 MHz) : 1.09 dt (J=7 & 12.5 Hz, H-2b), 1.19 s (H-14), 1.25 d (J=16.5 Hz, H-13), 1.69 m (H-15), 1.79 dd (J=5 & 13 Hz, H-9a), 1.88 dd (J=11.5 & 13 Hz, H-9b), 2.40 dt (J=12.5 & 7.5 Hz, H-2a), 2.48 dq (J=7 & 13 Hz, H-11), 2.79 dd br (J=7 & 7.5 Hz, H-1), 2.70 ddd (J= 4.5, 7 & 13 Hz, H-7), 4.39 t br (J=7 Hz, H-3), 4.64 d (J=4.5 Hz, H-6) and 5.02 ddd (J=5, 7 & 11.5 Hz, H-8). MS:m/z at 282 (M^+), 264, 246, 178, 156, and 108. MS CI (isobutane) : m/z at 283, 265 and 247.

EPOXIDATION OF 27 :

To a solution of 50 mg of 27 in 2 ml of dry dichloromethane was added 100 mg of MCPBA and stirred for 14 hours. The reaction mixture was then worked up as usual and the residue was purified by preparative TLC (EtlAc:Pet.ether, 4:1) to furnish 45 mg of the epoxide 31 as a gum. IR (cm^{-1}) : 3500, 1776, 1600, 1145 and 990; ^1H NMR 1.29 d (J=7 Hz, H-13), 1.36 s (H-14), 1.49 s (H-15), 1.92 dd (J=17 & 5 Hz, H-9a), 2.64 ddd(J=4.5, 7 & 13Hz,H-7), 3.7 m (overlapping signals of H-3 & H-6) and 4.50 m (H-8). MS:m/z at 308 (M^+), 292, 291, 277, 264 and 257. ^1H NMR of (31 + TAI) (60 MHz) 1.29 d (H-13), 1.49 s (H-15), 1.60 s (H-14), 3.2 ddd (H-7), 4.5-5.5 (overlapping signals of H-3, H-6 & H-8).

REACTION OF 31 WITH $BF_3.Et_2O$:

A solution of 40 mg of 31 in 2 ml dry diethyl ether and 0.5 ml dioxane was chilled to 0°C and treated with 3 drops of freshly distilled (over CaH_2) $BF_3.Et_2O$ The reaction mixture was stirred at 0°C for 15 min and then left in the dark at room temperature. After 12 hours it was worked up as described earlier. The residue showed two major spots on TLC, which were isolated by preparative TLC (EtOAc:Pet. ether, 4:1) to furnish 20 mg of 29 as a gum and 16 mg of 32 as a gum.

Spectral data of 29 : IR (cm^{-1}) : 3500, 2900, 1775, 1705, 1475, 1390, 1200, 1175, 1140, 1060, 1040 and 990; ^1H NMR (Assignment by DDS) : 1.35 d (J=7 Hz, H-13), 1.42 s (H-14), 1.56 dd (J=12 & 14 Hz, H-9a), 1.75 d (J=2 Hz, H-15), 1.98 dd (J=5 & 18 Hz, H-2a), 2.05 ddd (J=1, 4.5 & 14 Hz, H-9b), 2.14 dq (J=7 & 14 Hz, H-11), 2.71 dd (J=6.5 & 18 Hz, H-2b), 2.92 ddd (J=3.5, 7.5 & 14 Hz, H-7), 3.25 m (H-1), 5.02 d br (J=3.5 Hz, H-6),and 5.12 ddd (J=12, 4.5 & 7.5 Hz, H-8); MS:m/z at 280 (M^+), 279, 263, 262, 244, 222, 219, 207, 204, 167 and 149. Mass calcd. for $C_{15}H_{20}O_5$ (M^+),is 280.1095; Found 280.1099. Acetylation of compound 29 gave compound 30. Spectral data of 30 : IR (cm^{-1}) : 3500, 2900, 1775, 1740, 1710, 1475, 1375, 1200, 1175 and 990; ^1H NMR: 1.17 s br (H-14), 1.37 dd (J=6.5 & 18.5 Hz, H-2a), 1.39 d (J=7 Hz, H-13), 1.80 d (J=2 Hz, H-15), 1.87 d (J=10 & 14 Hz, H-9a), 2.18 (acetate), 2.20-2.30 (overlapping signals of H-9b and H-11), 2.62 dd (J=6.5 & 18.5 Hz, H-2b), 2.92 ddd (J=5.5, 8 & 11 Hz, H-7), 3.10 ddd (J=2, 3 & 6.5 Hz, H-1), 5.08 ddd (J=4, 8 & 10 Hz, H-8) and 6.10 d br (J=5.5 Hz, H-6); ^1H NMR (C_6D_6) (270 MHz) : 0.71 s (H-14), 0.98 d (J=6.5 Hz, H-13), 1.07 dd (J=10 & 15 Hz, H-9a), 1.52 d (J=2.5 Hz, H-15), 1.54 (acetate), 1.66 dd (J=4.5 & 15 Hz, H-9b), 1.81 dd (J=18.5 & 4.0 Hz, H-2a), 1.82 dq (J=7 & 11.5 Hz, H-11), 2.13 dd (J=18.5 & 6.5 Hz, H-2b), 2.49 ddq, br (J=2.5, 4.0 & 6.5 Hz, H-1) and 5.70 d br (J=5.5 Hz, H-6, H-8). MS:m/z at 322 (M^+) 280 and 262. Calcd. for $C_{17}H_{22}O_6$: mol.wt. 322.1414. Found (MS) 322.1426.

As compound 32 was found to be unstable, its spectral data could not be recorded.

ACETYLATION OF 32 :

To a solution of 15 mg of 32 in 1 ml of dry pyridine was added 1 ml acetic anhydride and left in the dark. After the overnight reaction, it was worked up as usual and purification of the crude mass by preparative TLC (EtOAc:Pet.ether,1:1) furnished 14 mg of 33 as a gum. IR (cm^{-1}) : 3500, 2900, 1775, 1735, 1715 and 1125; ^1H NMR: 1.35 s (H-14), 1.39 d (J=7 Hz, H-13), 1.80 d br (J=2 Hz, H-15), 2.08 s (acetate), 2.18 s (acetate), 2.79 dd br (J=4 & 15 Hz, H-9a), 2.93 m (H-11), 3.91 m (H-8), 5.09 dt (J=4 & 9 Hz, H-3) and 6.01 d br (J=6 Hz, H-6). MS:m/z at 364 (M^+), 336, 321, 320 and 305.

ACETYLATION OF 27 :

70 mg of 27 was dissolved in 2 ml of dry pyridine and 2 ml of acetic anhydride was added to it. After four hours, the reaction mixture was worked up as usual and the residue was purified by preparative TLC (EtOAc:Hexane, 1:1) to furnish 55 mg of 28 m.p. 125°C (EtOAc); IR (cm^{-1}) : 3500, 2900, 1775, 1740, 1450, 1375, 1250, 1160, 1025, 1000 and 975; ^1H NMR: 1.17 s br (H-14), 1.35 d (J=7 Hz, H-13), 1.75 dd (J=1 & 2 Hz, H-15), 1.83 dd (J=10.5 & 13.5 Hz, H-9a), 2.02 dd br (J=4.5 &

13.5 Hz, H-9b), 2.08 (acetate), 2.13 s (acetate), 2.40 dq (J=6.5 & 12.5 Hz, H-11), 2.63 ddd (J=4.5, 6.5 & 13 Hz, H-2a), 2.75 ddd (J=4, 7.5 & 12.5 Hz, H-7), 2.80 m (H-1), 5.09 ddd (J=4.5, 7.5 & 10.5 Hz, H-8), 5.48 qt br (J=1 & 6.5 Hz, H-3), 5.93 d (J=4 Hz, H-6). MS:m/z at 366 (M^+ absent), 307, 293, 288, 279, 264, 263, 262, 259, 144 and 132. HRMS for m/z at 307 found 307.1530 (± 0.002). Calcd. 307.1539 for $C_{17}H_{33}O_5$ (M^+-CH_3COO).

REACTION OF 28 WITH MCPBA :

A solution of 105 mg of 28 in 5 ml of dry dichloromethane was treated with 210 mg of m-chloroperbenzoic acid and the reaction was monitored by TLC. After 12 hours, the reaction mixture was worked up as usual and the residue showed four major spots on TLC which were isolated by preparative TLC (EtOAc:Pet.ether, 1:2; three developments) to furnish 26 mg of 34 (m.p. 135°C, EtOAc-Hexane), 18 mg of 36 as a gum, 16 mg of 30 as a gum, 15 mg of 37 as a gum.

Spectral data of 34 : IR (cm^{-1}): 3500, 2950, 1780, 1750, 1740, 1460, 1375, 1225, and 1050; ^1H NMR (Assignment by COSY 45 spectrum): 1.22 s (H-14), 1.34 d (J=7 Hz, H-13), 1.44 s (H-15), 1.70 ddd (J=1.5, 4 & 15 Hz, H-2a), 2.10 s (acetate), 2.20 s (acetate), 2.00-2.30 (overlapping signals of H-2b, H-9a & H-9b), 2.52 dq (J=12.5 & 7 Hz, H-11), 2.62 dd (J=4 & 10 Hz, H-1), 2.80 ddd (J=5.5, 9 & 12.5 Hz, H-7), 5.08 dt (J=4.5 & 9 Hz, H-8), 5.14 dd (J=1.5 & 6.5 Hz, H-3) and 5.32 d (J=5.5 Hz, H-6). MS:m/z at 382 (M^+), 365, 340, 323, 322, 280, 263, 262, 247, 244, 219, 207, 193, 173, 164, 148, 124, 95 and 43. (+) FAB MS:m/z at 405.3 $[(M+Na)^+]$, 383.3 $[(M+H)^+]$ 365.3, 323.3, 305.2, 279.2, 263.2, 245.2, 235.2, 214.1, 203.2, 189.2, 175.2, 161.2 149.2, 147.2, 133.2, 123.2, 109.2, 91.1, 81.1 and 69.1. The measured mass for $(M+H)^+$ ion in the (+) FAB spectrum is 383.1635, calcd. for $C_{19}H_{27}O_8$ is 383.1705.

Spectral data of 36 : IR (cm^{-1}): 3580, 3040, 2980, 1775, 1745 (very strong band), 1450, 1400, 1325, 1250, 1225 and 1050; ^1H NMR (Assignment by COSY 45 spectrum) : 1.18 d (J=7 Hz, H-13), 1.52 s (H-14), 1.66 s (H-15), 2.00 (H-2a), 2.15 s (two acetates), 2.20 (H-9a), 2.25 (H-9b), 2.35 (H-2b), 2.45 (H-1), 2.80 (H-7, H-11), 4.92 m (H-8), 5.26 dd (J=7 & 11 Hz, H-3) and 5.68 d (J=9 Hz, H-6). MS:m/z at 382 (M^+), 339, 304, 280, 262, 247, 95 and 43.

Spectral data of 37 : IR (cm^{-1}): 3500, 1775-1725, 1450, 1425, 1375, 1250, 1200 and 1050; ^1H NMR: 1.35 d (J=7 Hz, H-13), 1.43 s, 1.45 s (H-14 & H-15), 2.05 s (acetate) 2.15 s (acetate), 1.90-2.20 (overlapping signals of H-2a, H-9a & H-9b), 2.60 dd (J=7 & 16 Hz, H-2b), 2.70 dd (J=5.5 & 7 Hz, H-1), 2.74 dq (J=7 & 12 Hz, H-11), 2.85 m (H-7), 4.67 d (J=7 Hz, H-6), 5.00 ddd (J=4.7, 7.7 & 10.8 Hz, H-8) and 5.25 d br (J=11.4 Hz, H-3). MS:m/z at 399 (M^+-H), 356, 341, 339, 338, 223, 298, 297, 296, 295, 281, 280, 279, 263, 262, 261, 244, 243, 231, 222, 199, 182, 160, 142 and 43. HRMS for m/z at 341 found 341.1585 (± 0.002). Calcd. 341.1593 for $C_{17}H_{25}O_7$.

SODIUM BOROHYDRIDE REDUCTION OF 34 :

A solution of 20 mg of compound 34 in 2 ml of methanol was cooled to 0°C and treated with 25 mg of sodium borohydride. The reaction mixture was stirred at 0°C for 10 min. It was then worked up as usual and the residue furnished 12 mg of 35 as a gum by preparative TLC (EtOAc:Pet.ether, 2:1). IR (cm^{-1}): 3500, 1780, 1750, 1460, 1375, 1225 and 1050; ^1H NMR (90 MHz): 1.10 d (J=7 Hz, H-13), 1.18 s (H-14), 1.36 s (H-15), 2.00 s (acetate), 3.50 d br (J=11 Hz, H-4), 4.70 m (H-8), 4.80 m (H-3) and 5.25 d (J=4.5 Hz, H-6). MS:m/z at 384 (M^+), 367, 341, 340, 325, 266 and 43. ^1H NMR of (35 + TAI) (60 MHz): 1.35 s (H-14), 1.45 s (H-15), 4.90-5.20 (overlapping signals of H-3, H-4, H-6 and H-8) and 8.30 s br (two NH).

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