Tetrahedron Vol. 42, No. 4, pp. 1157 to 1167, 1986
Printed in Great Britain.

0040-4020/86 $3.00 + .00
Pergamon Press Ltd.

ABSOLUTE STEREOCHEMISTRY OF THE CADINENES FROM EUPATORIUM TRAPEZOIDEUM

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(*Received in UK* 14 *November* 1985)

Summary : Chemical examination of Eupatorium trapezoideum has furnished five cadinenes 1a, 2a, 2b, 4a & 5 and a degraded cadinene 3a whose stereostructures are presented in this paper. The absolute stereochemistry of the major component 1a has been determined by application of Horeau's method as well as chemical correlation studies and has been confirmed by X-ray analysis of 1f thus suggesting that it belongs to the amorphane group.

In continuation of our studies on the family Compositae in search of anticancer sesquiterpene lactones we examined the above ground parts of Eupatorium trapezoideum[1] Kunth. (Syn. E. adenophorum Kunth.) and isolated five cadinenes 1a, 2a, 2b, 4a & 5 and a degraded cadinene 3a. When the chemical examination of E. trapezoideum was in progress in our laboratory, Bohlmann et al reported the isolation of six cadinenes from Ageratina adenophora[2], three of them appeared to be identical (IR, NMR & MS) with 1a, 2a and 5. Structures of 1a and 2a were established purely on the basis of spectral data and we present chemical evidence which further support their structural assignments. In addition, the structures of two new cadinenes 2b, 4a and a new degraded cadinene 3a, are also delineated in this paper. Application of Horeau's method and chemical correlation studies suggested that the cadinenes from the title plant belong to the amorphane group of compounds[3] and this has been confirmed by X-ray analysis of 1f.

Compound 1a was obtained as an oil and analysed for $C_{15}H_{24}O_2$. In the IR spectrum the absorption peak at 3500cm^{-1} suggested the presence of a hydroxyl group which was confirmed by making the monoacetate 1b. IR spectrum of 1a also displayed an absorption band at 1670cm^{-1} indicating the presence of an α,β-unsaturated ketone group which was confirmed as follows. Hydrogenation of 1a over 10% Pd/C gave 6 in whose IR spec. the ketone band appeared at 1700cm^{-1}. Sodium borohydride reduction of 1a gave 1d which on acetylation furnished the diacetate 1e. Hydrogenation of 1d over 10% Pd/C furnished 7.

The following chemical evidence established the location of the hydroxyl group at C-8. Reaction of 1a with mesyl chloride in pyridine furnished the

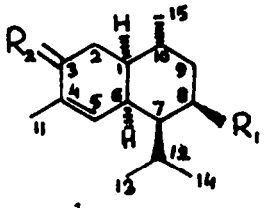
monomesylate 1c which on heating with dry DMSO at 100°C yielded 8 in a poor yield. Reaction of 1a with $POCl_3$ in pyridine furnished 8 in 70% yield which on refluxing in benzene containing a crystal of toluene-p-sulphonic acid furnished the dienone 9a in 65% yield.

Oxidation of 1a with Jones reagent furnished a compound which was identical (IR, NMR, Mass) with 2a. Isomerization of 2a on acidic alumina (activity II) furnished 2b and vice versa thus suggesting that they are epimeric at C-7[4]. Reduction of 2a with sodium borohydride gave 1a as the major product whereas a similar reduction of 2b gave 2c which on acetylation formed the monoacetate 2d. In the NMR spectrum of 2c & 2d, H-8 appeared as a broad multiplet ($W_{\frac{1}{2}}$ = 16Hz) in conformity with its orientation as axial. Most probably 2b is not an artifact of 2a because TLC (SiO_2) examination of the crude obtained by cold extraction of the plant material indicates the presence of both 2b and 2a.

Compound 4a was obtained as an oil and analysed for $C_{15}H_{24}O_3$. The absorption peak at 3400 and 1715cm^{-1} in the IR spec. suggested the presence of a hydroxyl and a ketone group in it. That the hydroxyl group was tertiary and α to the ketone became obvious from the acetylation behavior (Ac_2O/Py, 100° for 8 hr) of 4a to afford the monoacetate 4b in whose NMR spectrum the methyl singlet present at 1.25 ppm in 4a underwent paramagnetic shift to 1.40 ppm. Since the signals at 4.04 ppm (d, J=5Hz) and 3.86 ppm (1H multiplet) were not affected during acetylation, it was concluded that they represent the protons under an ether oxygen. At this stage, a fortuitous discovery established its correlation with 1a as follows. Osmium tetroxide/$NaIO_4$ oxidation of 1d furnished a product which was found to be identical with 4a in every respect (TLC, IR, NMR & Mass). The combination of OsO_4 & $NaIO_4$ has been reported to oxidise the secondary hydroxyl function[5]. Sodium borohydride reduction of 4a gave 4c which furnished the monoacetate 4d on acetylation and gave back the ketone 4a on oxidation with Collins reagent.

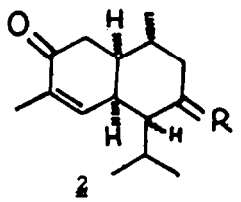
Compound 3a was obtained as an oil and analysed for $C_{12}H_{16}O_2$, thus suggesting the presence of only twelve carbon atoms in it which was confirmed by ^{13}C NMR spectrum in which they appeared at 209.59 *s*, 198.30 *s*, 146.19 d, 135.80 *s*, 46.59 t, 44.50 t, 41.37 d, 40.49 t, 37.92 d, 32.70 d, 19.91 q and 15.60 q. In the IR spectrum the bands at 1715 and 1670cm^{-1} suggested the presence of two keto groups and one of them as an α,β-unsaturated ketone, which was also evident from the ^{13}C NMR spectrum (a singlet at 198.30 ppm) given above. In the ^1H NMR spectrum (360 MHz)[6] the presence of two multiplets at 6.48 and 3.20 ppm, each integrating to one proton, a methyl on the double bond at 1.76 ppm and a methyl doublet at 1.04 ppm suggested the absence of an isopropyl group in it. The multiplicity of the three methylene groups appearing at 2.10-2.80 ppm suggested that each of them is coupled to only one proton besides geminal coupling. On the basis of above data, due to close resemblence of its ^1H and ^{13}C NMR spectra with that of 2a and 2b and decoupling experiments, structure 3a was assigned to this degraded cadinene[7]. Sodium borohydride reduction of 3a in the presence of $ZnCl_2$ furnished a mixture of two compounds which were identified as 3b & 3c.

Relative stereochemistry : The relative stereochemistry to 1a was assigned on the basis of coupling constants (See experimental) obtained through decoupling experiments on the ^1H NMR spec. of 1a recorded in C_6D_6 in which case the signals were well separated as compared to its spectrum in $CDCl_3$[8]. Since cis decalin can exist in two conformations it is clear from the coupling constants $J_{5,6}$= 2.0 Hz and $J_{1,6}$= 5 Hz that H-6 is equatorial and H-1 is axial. The large value of coupling constant between H_1 and H_{10} ($J_{1,10}$= 10 Hz) suggests that the methyl group at C-10 is equatorial. The correlation between 1a and 4a establishes the stereochemistry of the latter at all the centres except



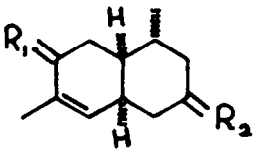
1

(a) R_1 = OH, R_2 = O
(b) R_1 = $OCOCH_3$, R_2 = O
(c) R_1 = MsO, R_2 = O
(d) R_1 = OH, R_2 = H, OH
(e) R_1 = $OCOCH_3$, R_2 = H, $OCOCH_3$
(f) R_1 = $OCOC_6H_4(Br)$-p, R_2 = O



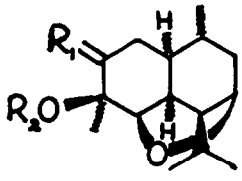
2

(a) H-7α, R = O
(b) H-7β, R = O
(c) H-7β, R = α-OH, H
(d) H-7β, R = α-$OCOCH_3$, H



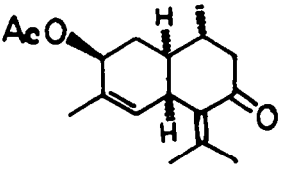
3

(a) R_1 = O, R_2 = O
(b) R_1 = O, R_2 = H, OH
(c) R_1 = H, OH; R_2 = H, OH

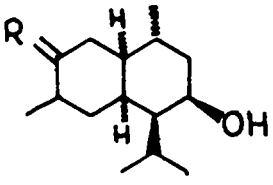


4

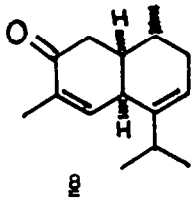
(a) R_1 = O, R_2 = H
(b) R_1 = O, R_2 = $-COCH_3$
(c) R_1 = H, OH; R_2 = H
(d) R_1 = H, $OCOCH_3$, R_2 = H



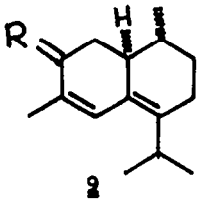
5



6 R = O
7 R = H, H

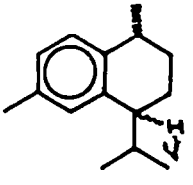


8



9

(a) R = O
(b) R = H, OH
(c) R = H, OTMS
(d) R = H, H



10

(a) H-7β
(b) H-7α

C-4 which is assigned on the basis of the fact that OsO_4 oxidation yields cis diol and the linkage between C-5 and C-8 is possible only when H-6 is equatorial and the hydroxyl at C-8 is axial (molecular models).

Absolute stereochemistry :

The absolute configuration of C-8 in 1a was established as R by application of Horeau's method[9]. Reaction of 1a with (±)-α-phenylbutyric anhydride in pyridine furnished (+)-α-phenylbutyric acid in 14% optical yield. Since the relative stereochemistry at C_1, C_{10} and C_6 through C_8 was known, 1a represents the absolute stereochemistry. This conclusion was further reinforced by the CD spectra of 1a and 6 and chemical correlation studies described below.

Sodium borohydride reduction of 2a furnished 2b in 80% yield. All attempts at the reductive removal of the hydroxyl in 2b with $LAH/AlCl_3$ furnished a residue as an oil, which appeared to be homogeneous on TLC but whose ^1H NMR spec. indicated it to be a mixture of calamenenes 10a and 10b[10]. The reductive removal of the hydroxyl in 2b was finally achieved by nickel boride[11] reduction of 9c when ent-10-epizonarene 2d, $[\alpha]_D$ +80°, (reported[12] $[\alpha]_D$ +175°) was obtained in 90% yield[13].

Finally the p-bromobenzoate derivative 1f provided suitable crystals for X-ray crystallographic analysis which established the absolute stereochemistry of 1a as depicted in the structure thus suggesting that the cadinenes from E. trapezoideum can be classified as amorphanes[14]. Compound 1a exhibits appreciable antifeedant action against 4th-instar caterpillars of the Eri Silk worm (Philosomia ricini Hutt).

X-Ray Crystallography

X-Ray diffraction data were collected on a Syntex $P2_1$ diffractometer from crystal of dimensions 0.5 x 0.5 x 0.6 mm. $C_{22}H_{27}BrO_3$, M=419.37, Triclinic, P1, with a=10.465(2), b=11.061(3), c=9.480(2) Å, α=100.58(2), β=105.43(2), γ=84.41(3)°, V=1038.4(5) Å^3, D_x=1.34gcm^{-3} for Z=2, MoK_α, λ=0.71093 Å, μ=1.97mm^{-1}. 6863 reflections were measured in eight octants of which 4289 had $I \geq 1.96\sigma(I)$ and were considered observed. Semiempirical absorption corrections based on ψ scan of eight reflections were applied.

The structure was solved with DIRDIF[15] with Br-atom positions obtained from Patterson synthesis. In the space group P1 the choice of origin is arbitrary and for convenience was assumed coincident with one of the bromine atoms. Positions of the H-atoms were calculated geometrically. Molecular model was refined by block-diagonal least-squares technique with non-H atoms anisotropic and all but methyl H-atoms as "riders"; methyl groups were treated as rigid groups. All H-atoms were assigned a common temperature factor which refined to U=0.107 Å^2. Function minimized $\sum w(|F_o| - |F_c|)^2$, $w=4.7251/(\sigma^2_{F_o} + 0.003F^2_o)$.

To determine the absolute configuration a model without f" corrections was refined. The parameters obtained were used in two structure factor calculations without and with inverted signs of f" corrections[16]. The R (and wR) values of 0.080 and 0.052 (0.126 and 0.073) obtained respectively for the two models indicated that the latter was the correct one. Therefore the signs of all atomic coordinates were changed to "-" and the refinement process was continued until it reached a convergence. Final R = 0.050 (wR = 0.070) for 4286 reflections. Refinement was carried out using SHELX 76 program[17], molecular geometry was studied using programs included in CRYPOZ library[18] and ORTEP[19] was used to prepare drawings.

There are two independent molecules (hereinafter molecules I and II) in the asymmetric unit cell. The overall conformation and the absolute configuration of

both molecules is illustrated in Fig. 1. The cis-decalin system is of non-steroid type with the C(1)-H(1) and C(6)-H(5) bonds in an α-orientation. The equatorial substituents at C(7) and C(10) are β-and α-oriented, respectively, and the benzoyloxy substituent at C(8) is axial and β. This classifies the investigated compound to the amorphane class of cadinene group of sesquiterpenes.

The conformations of the two molecules are fairly similar. The cyclohexane rings show essentially identical slightly flattened chair conformations (the averages of the endocyclic torsion angle magnitudes are 52.9(0.8) and 51.8(1.8)° for I and II, respectively). The cyclohexenone rings show some small conformational differences; the cyclohexenone ring of I adopts a distorted C(1)-sofa conformation, while that of II is slightly more puckered and adopts a conformation intermediate between C(1)-sofa and C(1), C(2) half-chair. Substantial difference in the torsion angles C(7)-C(8)-O(2)-C(16) of 22.1° indicates that the two molecules differ slightly in the orientation of the benzoyloxy substituent at C(8).

Average and maximal differences in bond lengths between the two molecules are 0.025(18) and 0.075(13) Å, and in bond angles 1.8(1.5) and 5.6(7)°. The estimated standard deviations of bond lengths and angles are large and it would be of no significance to account for small variations in the geometrical parameters. However, there are several bond distances and angles which differ between two molecules by more than 3 σ and these might deserve a comment. The largest differences between I and II occur in the geometry of the isopropyl and p-bromophenyl substituents and within the α,β-unsaturated ketone group. The differences in the bond lengths in the A ring are such that they might be ascribed to the higher degree of electron delocalization in the conjugated $-\overset{\beta}{C}-\overset{\alpha}{C}=C-$ system in I as compared with the more localized bonds in II. In the phenyl rings, apart from individual differences, the average bond distances and angles are equal in both molecules. The average bond lengths are 1.386(25) and 1.385(15) and the average intra-ring angles are 120.2(1.4) and 120.0(1.4)° for molecules I and II, respectively.

The phenyl rings in both molecules are virtually planar, although the least-squares plane through the phenyl ring is less rigorous in I. The in-plane atoms deviate by no more than 0.019(6) and 0.007(8) Å, with the Br atoms displaced by 0.082 and 0.008(1) Å in molecules I and II, respectively.

There is no possibility for hydrogen bonding in the crystal. Inspection of the intermolecular contacts, however, revealed a distance between the carbonyl oxygen atom O(1) and the bromine atom Br'at 1+x,y,1+z of only 3.217(6) Å, significantly less than the sum of the van der Waals radii of the corresponding atoms. Such short C-Br ... O contacts have also been observed in steroids whose structures were determined as bromine derivatives, where they appear to dominate the molecular packing[20]. In the present case the question arises as to whether the observed C-Br ... O interaction is strong enough to account for small but significant differences in geometrical parameters between the two molecules. These differences may as well reflect a slight underestimation of the e.s.d.'s, a slight difference in thermal motions or a combination of all of these[21].

EXPERIMENTAL

Melting points were determined on Büchi oil heating type melting point apparatus and are uncorrected. IR spectra were determined in $CHCl_3$ on Perkin Elmer 237B spectrophotometer. The NMR spectra were recorded at 60 MHz (τ-60) in $CDCl_3$ unless otherwise stated with TMS as external standard. Chemical shifts are expressed as δ in ppm. Mass spectra were recorded under electron impact at 70 ev on MS-30 spectrometer. UV spectra were recorded on Beckmann spectrophotometer-26 in MeOH. Rotations were recorded on Jasco DIP-180. For preparative TLC Silica gel G (BDH, India) was used. Petroleum ether refers to the fraction b.p. 60-80°.

Extraction of Eupatorium trapezoideum :

Above ground parts of Eupaterium trapezoideum Kunth. (Syn. E. adenophorum) (1.8 kg), collected from Shillong, Meghalaya in February 1981 were shade dried and extracted in a soxhlet with chloroform till the extract was colourless. Solvent was evaporated at reduced pressure and the residue dissolved in 200 ml of methanol to which 20 ml of water was added and left overnight at r.t. The precipitated material was filtered out and the filtrate was extracted with pet ether (7x200 ml). The aqueous methanol layer was concentrated at reduced pressure when most of the methanol was removed. The aqueous residue was then extracted with chloroform (8x200 ml), washed with water and dried. Evaporation of the solvent under reduced pressure left 17.0 g of a gummy residue which was chromatographed over 500 g of acidic Al_2O_3 (activity II) column packed in benzene and 200 ml fractions being collected in the following order :

Fr. 1-2 (Bz), Fr. 3-6 (Bz:$CHCl_3$, 3:1), Fr. 7-10 (Bz:$CHCl_3$, 2:1), Fr. 11-12 (Bz:$CHCl_3$, 1:1), Fr. 13-14 ($CHCl_3$), Fr. 15-16 ($CHCl_3$:MeOH, 99:1), Fr. 17-19 ($CHCl_3$:MeOH, 95:5), Fr. 20-21 ($CHCl_3$:MeOH, 90:10), Fr. 22 (MeOH).

Fr. 5-10 showed two major spots on TLC which were combined (1.29 g) and separated by preparative TLC (Bz:pet-ether, 4:1) developing the plate six times. The less polar material 2a was obtained as a gum, yield 300 mg, $[\alpha]_D^{25}$ + 156° (c, 0.5 in $CHCl_3$). IR: 1705, 1660, 1600, 1175, 1100 and 1025cm^{-1}; NMR: 6.30 m (H-5), 3.30 m (H-6), 1.60 m (H-11), 0.90 (overlapping signals of H-13, H-14 and H-15); NMR (C_6D_6): 6.27 m (H-5), 1.72 m (H-11), 1.02 d (J=6.0 Hz, H-15), 0.72 d, 0.60 d (J=6.5 Hz, H-13, H-14); ^{13}C NMR: 210.44 s, 197.81 s, 141.36 d, 136.64 s, 60.25 d, 50.28 d, 45.19 d, 43.72 d, 41.72 d, 33.27 t, 23.30 t, 22.48 q, 19.94 q, 19.02 q, 15.71 q; MS:m/z 234 (M^+), 192, 150, 149, 136, 135. The more polar material 2b was also obtained as a gum, yield 200 mg, $[\alpha]_D^{25}$ + 52° (c, 0.5, $CHCl_3$); IR: 1700, 1670, 1600, 1100 and 975cm^{-1}; NMR: 6.27 m (H-5), 3.00 m (H-6), 1.60 m (H-11), 0.80 (overlapping signals of H-13, H-14 & H-15); NMR (C_6D_6): 6.10 m (H-5), 1.66 m (H-11), 0.80 (overlapping signals of H-13, H-14 & H-15); MS:m/z 234 (M^+), 192, 150, 144, 136, 135. MS:m/z calc. for $C_{15}H_{22}O_2$: 234.1618. Found 234.1604.

Fr. 13-18 (0.64 g) were again a mixture of two compounds which were separated on preparative TLC (Bz:EtOAc, 9:1) by developing the plate twice. The less polar material, yield 200 mg was identified as 2b and the more polar material obtained as a gum, yield 80 mg, was identified as 1a which was present in Fr. 19 as the major compound.

Fr. 19 (3.26 g) showed several spots on TLC and 1a as the major component was rechromatographed on 75 g of acidic Al_2O_3 (activity grade II) and 150 ml fractions were collected in the following order :

Fr. 1-10 (Bz), Fr. 11-20 (Bz:$CHCl_3$, 20:1), Fr. 21-30 (Bz:$CHCl_3$, 10:1), Fr. 31-40 (Bz:$CHCl_3$, 5:1), Fr. 41-50 (Bz:$CHCl_3$, 3:1), Fr. 51-60 (Bz:$CHCl_3$, 1:1), Fr. 61-70 (Bz:$CHCl_3$, 1:2), Fr. 71-80 (Bz:$CHCl_3$, 1:5), Fr. 81-90 (Bz:$CHCl_3$ 1:10), Fr. 91-100 ($CHCl_3$), Fr. 101-110 ($CHCl_3$:MeOH, 99:1), Fr. 111-120 ($CHCl_3$:MeOH, 95:5), Fr. 121-130 ($CHCl_3$:MeOH, 90:10), Fr. 131-140 (MeOH).

Fr. 98-110 furnished 1.2 g of pure 1a as an oil, $[\alpha]_D$ + 48° (c, 0.5, $CHCl_3$); CD (MeOH), $\Delta\epsilon_{285}$ + .22, $\Delta\epsilon_{220}$ + .64; IR: 3500, 1670, 1195, 1095, 1030, 980 & 960cm^{-1}; ^1H NMR (270 MHz in $CDCl_3$): 6.63 s br (H-5), 4.15 ddd (H-8), 2.96 m (H-6), 2.82 dd (H-2), 2.48 dd (H-2'), 2.08 m (H-12), 1.76 m (H-11), 1.15 d (H-15), 1.06 d (H-13), 0.90 d (H-14); ^1H NMR (400 MHz in C_6D_6): 6.68 ddq ($J_{1,5}$= $J_{5,6}$= 2 Hz, $J_{5,11}$= 1.5 Hz, H-5), 3.78 ddd ($J_{7,8}$= $J_{8,9}$= $J_{8,9}$= 2.7 Hz, H-8), 2.74 dd ($J_{2,2'}$ = 16, $J_{1,2}$= 2.5 Hz, H-2), 2.55 dddq ($J_{1,6}$= 5, $J_{6,7}$= 4 & $J_{6,11}$= 2 Hz, H-6), 2.11 dd ($J_{1,2}$= 4.5 Hz, H-2'), 1.19 dddd ($J_{1,10}$= 10 Hz, H-1), 1.88 dd (H-11), 0.93 d (J=6.5 Hz, H-13, H-14), 0.81 d (J=6 Hz, H-15); ^{13}C NMR ($CDCl_3$); 199.76 s, 147.72 d, 133.28 s, 67.24 d, 51.80 d, 45.67 d, 42.91 d, 42.60 d, 37.49 d, 25.19 t, 23.03 t, 21.82 q, 20.64 q, 19.18 q, 15.98 q. MS:m/z at 236 (M^+), 218 & 175. Found C 76.58; H 10.20, $C_{15}H_{24}O_2$ requires C 76.23; H, 10.24%.

Fr. 111-118 (0.60 g) were a mixture of two compounds which were separated by preparative TLC. The less polar material was identified as 1a, 0.20 g and the more polar material (0.25 g) obtained as a gum was identified as 4a $[\alpha]_D$ + 30° (c, 0.33 in CCl_4). IR: 3400, 1715, 1075, 1025, 1010, 975, 955, 900, 870, 850 & 790cm^{-1}; NMR: 4.04 d (J=5 Hz, H-4), 3.86 m (H-8), 1.25 s (H-11), 1.00 (9 protons, overlapping signals of H-13, H-14 & H-15); MS:m/z at 252 (M^+), 234 (M^+-H_2O, base peak), 218, 209, 191 (M^+-H_2O-C_3H_7). MS:m/z calc. for $C_{15}H_{24}O_3$: 252.1724. Found 252.1710.

Evaporation of the petroleum ether extract furnished 15 g of the crude product which was chromatographed on 500 g of acidic aluminium oxide (activity II) and 200 ml fractions were collected in the following order:

Fr. 1-15 (Benzene), 16-20 (Bz:EtOAc, 100:1), 21-25 (Bz:EtOAc, 50:1), 26-30 (Bz:EtOAc, 25:1), 31-38 (Bz:EtOAc, 20:1), 39-42 (Bz:EtOAc, 15:1), 43-47 (Bz:EtOAc, 10:1), 48-57 (Bz:EtOAc, 5:1), 58-60 (EtOAc).

Fr. 18 (0.258 g) was purified by preparative TLC (pet-ether:EtOAc, 10:1) to furnish 5, yield 0.15 g, as an oil and identical IR, NMR & Mass spectra with that reported in the literature[22].

Fr. 46–49 showing single spot on TLC were combined (1.28 g) and compound 1a was isolated as an oil by preparative TLC (Pet-ether:EtOAc, 4:1, 4 developments) yield 0.2 g; $[\alpha]_D^{25}$ + 49.6° (c, 2.5 in CCl_4); IR: 1715, 1670, 1225, 1130, 1105, 1080, 910 & 750cm^{-1}; NMR (360 MHz in $CDCl_3$): 6.48 m (1H), 3.20 m (1H), 2.75 dd (J=16, 7.5 Hz, 1H), 2.60 dd (J=16, 6 Hz, 1 H), 2.54 dd (J=18, 5 Hz, 1H), 2.45 dd (J=4.5, 12 Hz, 2H), 2.25 m (1H), 2.15 dd (J=18, 8 Hz, 1H), 2.08 m (1H), 1.76 m (3H), 1.04 d (J=6 Hz, 3H); ^{13}C NMR: 209.59 s, 198.30 s, 146.19 d, 135.80 s, 46.59 t, 44.50 t, 41.37 d, 40.49 t, 37.92 d, 32.70 d, 19.91 q & 15.60 q; MS:m/z 192 (M^+), 175, 160, 159. MS:m/z calc. for $C_{12}H_{16}O_2$: 192.1150. Found 192.1134.

Acetylation of 1a

A solution of 42 mg of 1a in 0.5 ml of pyridine and 1 ml of acetic anhydride was left overnight at r.t. The reaction mixture was worked up as usual and the residue purified by preparative TLC (pet-ether:EtOAc, 9:1) to furnish 42 mg of 1b as a gum; IR: 1720, 1670, 1600, 1200 & 1180cm^{-1}; NMR: 6.60 m (H-5), 5.08 m (H-8), 1.98 (acetate methyl), 1.78 m (H-11), 1.05 d (J=7 Hz, H-13, H-14 & H-15). Mass spec. m/z at 278 (M^+), 236, 218, 203 (M^+-AcOH-CH_3), 193 (M^+-C_2H_2O-C_3H_7) & 175 (M^+-$C_2H_4O_2$-C_3H_7); Found: C, 73.22; H, 9.14. $C_{17}H_{26}O_3$ requires C 73.35; H, 9.41%

Jones oxidation of 1a

A solution of 75 mg of 1a in 10 ml of acetone was treated at 0° with four drops of Jones reagent. After 35 mints. the reaction was quenched by adding 5 ml of MeOH to the reaction mixture followed by 100 ml of water. It was extracted with chloroform, washed with dilute $NaHCO_3$ solution and water. Evaporation of the dried extract at reduced pressure followed by purification of the residue on preparative TLC (pet-ether:EtOAc, 9:1) furnished 35 mg as a gum, identical in every respect (TLC, IR, NMR & Mass) with 2a.

Hydrogenation of 1a

A solution of 30 mg of 1a in 25 ml ethyl acetate was hydrogenated over 100 mg of Pd/C (10%) at atmospheric pressure for one hour. The reaction mixture was filtered and the catalyst washed thoroughly with ethyl acetate. The combined washings and the filtrate were evaporated under reduced pressure and the residue purified by preparative TLC (Bz:EtOAc, 9:1) to furnish 6, 30 mg, m.p. 124–126° (EtOAc); CD (MeOH), $\Delta\varepsilon_{285}$=-.68; IR: 3500, 1700, 1195 & 1025cm^{-1}; NMR: 4.10 m (H-8), 1.00 (12 protons, overlapping signals of H-11, H-13, H-14 & H-15); Mass spec. m/z 238 (M^+), 220, 205, 202, 177 (M^+-H_2O-C_3H_7), 162 (M^+-H_2O-CH_3-C_3H_7). Found: C, 75.36, H, 10.74. $C_{15}H_{26}O_2$ requires C 75.58; H, 10.99%.

$NaBH_4$ reduction of 1a

To a solution of 50 mg of 1a in 4 ml methanol was added 170 mg of sodium borohydride and the reaction mixture stirred at r.t. for 19 hr. Dilution with water was followed by extraction with chloroform (3x100 ml). The washed and dried extract was evaporated at reduced pressure and the residue showed one major spot on TLC which was separated by preparative TLC (Bz:EtOAc, 4:1) to furnish 1d as a gum (20 mg); IR: 3500, 1100, 1010, 960 and 925cm^{-1}; NMR: 5.60 m (H-5), 3.90 m (H-3 and H-8), 1.65 m (H-11), 0.98 d (J=6 Hz, H-13, H-14), 0.90 d (J=7 Hz, H-15); Mass spec.:m/z 238 (M^+), 220, 202 & 159 (M^+-$2H_2O$-C_3H_7). Found: C, 75.86, H, 11.12. $C_{15}H_{26}O_2$ requires C, 75.58; H, 10.99%.

Acetylation of 1d with acetic anhydride and pyridine gave the diacetate 1e as a gum. IR: 1725, 1200, 1010cm^{-1}; NMR: 5.60 m (H-5), 5.10 m (H-3 & H-8), 2.00 & 1.98 (acetate methyls), 1.65 m (H-11), 1.00 (9 protons, overlapping signals of H-13, H-14 & H-15); Mass spec.:m/z at 322 (M^+), 280, 260, 220, 202, 159 (M^+-2 $C_2H_4O_2$-C_3H_7).

OsO_4-$NaIO_4$ oxidation of 1d

A solution of 150 mg of 1d in 15 ml dioxane and 4 ml of water was treated with 20 mg of OsO_4 and the reaction mixture stirred at r.t. for one hr. 1.0 g of powdered $NaIO_4$ was added in portions over a period of 30 mints and the stirring continued for a further period of 15 hr. The reaction mixture was filtered and the filtrate diluted with 200 ml of dichloromethane. The washed and dried extract was evaporated at reduced pressure and the residue purified by preparative TLC (Bz:EtOAc, 3:1) to furnish a gum (60 mg) which was identical in every respect (IR, NMR and Mass spec.) with naturally occurring compound 1a.

Hydrogenation of 1d

A solution of 40 mg of 1d in 25 ml of ethyl acetate was hydrogenated over 10% Pd/C at atmospheric pressure for 10 hr. Usual work up procedure provided 35 mg of 7 as an oil. NMR: 4.00 m (H-8), 0.8–1.00 (4 methyls, overlapping

signals); Mass spec.:m/z at 224 (M^+), 206, 191, 163 & 148.

Reaction of 1a with $POCl_3$

A solution of 40 mg of 1a in 2 ml of dry pyridine was treated with 0.25 ml of phosphorus oxychloride at 0°C and the reaction mixture left at r.t. for 2 hr. Dilution with cold water was followed by extraction with dichloromethane (4x50 ml) which was washed with dilute $NaHCO_3$ solution and water. Evaporation of the dried extract yielded a residue which was purified by preparative TLC (pet-ether:EtOAc, 9:1) to furnish 26 mg of 8 as an oil. IR: 1675, 1190, 1100 and 910cm^{-1}; NMR: 6.40 m (H-5), 5.40 m (H-8), 1.70 m (H-11), 1.05 d (J=7 Hz, H-13, H-14 & H-15). Mass:m/z at 218 (M^+), 203 & 175.

Isomerization of 8 to 8a

A solution of 30 mg of 8 in 4 ml of dry benzene containing a crystal of toluene-p-sulphonic acid was refluxed on water bath for 1 hr. The reaction mixture was diluted with 200 ml of CH_2Cl_2 and washed with dil. sodium bicarbonate solution and water. Evaporation of the solvent furnished 28 mg of 8a as an oil. IR: 1650, 1120, 900 and 850cm^{-1}; NMR: 7.20 m (H-5), 1.80 m (H-11), 1.00 (overlapping signals of H-13, H-14 & H-15). MS:m/z at 218 (M^+), 203 & 175.

$NaBH_4$ reduction of 8a

A solution of 30 mg of 8a in 2 ml methanol was stirred with 20 mg $NaBH_4$ for one hr. Usual work up procedure followed by purification on preparative TLC (Bz:EtOAc, 9:1) provided 24 mg of 9b as an oil and as the major product. IR: 3500, 1100, 1050, 1000, 950 & 825cm^{-1}; NMR: 6.18 m (H-5), 4.08 m (H-3), 1.80 s br (H-11), 0.95 d (J=7 Hz, H-13, H-14 & H-15). Mass spec.:m/z at 220 (M^+), 202 (M^+-H_2O), 159 ($M^+-H_2O-C_3H_7$), 144.

Nickel boride reduction of 8c

A solution of 24 mg of 8b in 2 ml of hexamethyldisilazane was treated with 0.5 ml of chlorotrimethylsilane. After one hour when the reaction was complete (TLC), the solvents were distilled off under vacuum and the residue 8c was dissolved in 2 ml of dry diglyme and treated with 100 mg of $NiCl_2.6H_2O$ followed by 100 mg of sodium borohydride. The reaction mixture was stirred at r.t. monitoring on TLC. After 8 hr when no starting material was left, the reaction mixture was diluted with water (100 ml) and extracted with petroleum ether. Removal of pet-ether furnished a residue which was purified by preparative TLC (Petroleum ether) to yield 20 mg of 8d as an oil; $[\alpha]_D^{25}$ + 80° (reported[12] $[\alpha]_D$ + 175°); UV, λ_{max}^{243nm} (ϵ 12200); IR: 1650, 1610 & 860cm^{-1}; NMR: 6.18 s br (H-5), 3.10 m (H-1), 1.80 br (H-11), 1.0 d (J=7 Hz, H-13, H-14 & H-15); MS:m/z at 204 (M^+), 189 & 161 (base peak).

LAH-$AlCl_3$ reduction of 8b

A solution of 30 mg of 8b in 4 ml of dry ether was treated with 20 mg LAH and 50 mg $AlCl_3$. The reaction mixture was stirred at r.t. monitoring on TLC. After half an hour the reaction mixture was diluted with saturated ammonium chloride solution and extracted with ethyl acetate (3x100 ml). The washed and dried extract was evaporated and the residue purified on TLC (Pet-ether) to furnish 18 mg as an oil of the mixture of 10a & 10b; NMR: 6.90 s (3H), 2.20 s and 2.15 s (total integration, three protons, indication of mixture of 10a and 10b), 1.00 (overlapping signals of three methyl groups); MS:m/z 202 (M^+), 187 & 159. When a solution of 8b in benzene containing a crystal of toluene-p-sulphonic acid was refluxed for half an hour, the mixture of 10a and 10b was obtained in 50% yield.

Mesylation of 1a

A solution of 40 mg of 1a in 2 ml dry pyridine was treated with 0.25 ml of methanesulphonyl chloride and the reaction mixture left overnight at r.t. Usual work up procedure followed by purification of the crude product on preparative TLC (EtOAc:pet-ether, 1:9) furnished 20 mg of 1c as an oil. IR: 1680, 1180, 1150, 1100, 960, 920 & 900cm^{-1}; NMR: 6.60 m (H-5), 5.05 m (H-8), 2.80 s ($-O-\overset{O}{\overset{\|}{S}}-CH_3$), 1.75 m (H-11), 1.00 (9 protons, overlapping signals of H-13, H-14 & H-15); Mass spec.:m/z 218 (M^+-MsOH), 203 & 175.

Conversion of 1c to 8

A solution of 20 mg of the mesylate 1c in dry DMSO was kept at 100° for 2 hr. Usual work up procedure followed by separation of the crude product on preparative TLC (Pet-ether) furnished 5 mg of 8 as an oil.

$NaBH_4$ reduction of 2a

A solution of 60 mg of 2a in 2 ml of MeOH was cooled to -10° and treated with 50 mg of $NaBH_4$. The reaction mixture was stirred at -10° for 20 mint., diluted with water, acidified with dil. acetic acid and extracted with CH_2Cl_2 (5x100 ml). The washed and dried extract was evaporated and the residue purified by preparative TLC to furnish 20 mg of 1a as an oil which was found to be identical (IR, NMR and Mass) with the naturally occurring sample of 1a.

$NaBH_4$ reduction of 2b

A solution of 40 mg of 2b in 2 ml of methanol was cooled to -10°C and treated with 50 mg of $NaBH_4$. The reaction mixture stirred magnetically at -10°C for 30 mint. It was acidified with dilute acetic acid, diluted with water and then extracted with dichloromethane (5x100 ml). The washed and dried extract was evaporated and the residue purified by preparative TLC (Bz:EtOAc, 9:1) to furnish 2c, 20 mg as an oil. IR: 3500, 1670, 1075, 1040, 1020 & 900cm^{-1}; NMR: 6.40 m (H-5), 3.90 m ($W_{\frac{1}{2}}$ 16 Hz, H-8), 1.70 s br (H-11), 0.9-1.10 (9 protons); Mass spec.:m/z at 236 (M^+), 218, 203, 175, 162 & 147. Acetylation of 2c with Ac_2O/Py gave the acetate 2d as an oil; NMR: 6.55 m (H-5), 4.90 m ($W_{\frac{1}{2}}$ 16 Hz, H-8), 2.00 (acetate), 1.80 s br (H-11), 1.05 (9 protons). Mass spec.:m/z at 278 (M^+), 230, 218, 203, 193 and 175.

Isomerization of 2a to 2b and Vice versa

A solution of 50 mg of 2a in 0.5 ml of benzene was placed on a column of acidic alumina (50 g, activity II) and left for six days at r.t. Elution of the column with 10% MeOH in chloroform (200 ml) gave a mixture of two compounds which were separated by preparative SiO_2 TLC (Pet-ether) to furnish 18 mg of 2a and 28 mg of 2b. Similar experiment with 50 mg of 2b furnished 15 mg of 2a and 30 mg of 2b.

$NaBH_4$ reduction of 3a

A solution of 24 mg of 3a in 1.0 ml dry diglyme was cooled to 0°C and treated with 30 mg of $NaBH_4$ and 60 mg $ZnCl_2$. The reaction mixture was stirred at 0-5°C, monitoring the reaction on TLC. After 5 hr, the reaction mixture was diluted with water and extracted with dichloromethane (5x100 ml). The washed and dried extract was evaporated and the residue (21 mg) separated on preparative TLC (Bz:EtOAc, 5:1, 4 times development). The least polar band was obtained as an oil, yield 12 mg, and was identified as 3b; IR: 3500, 1670, 1000, 910cm^{-1}; NMR: 6.40 m (H-5), 3.80 m (H-8), 1.70 s br (H-11), 0.90 d (J=6.5 Hz, H-15); Mass spec.:m/z 194 (M^+), 176 & 161. The more polar band was also obtained as an oil, yield 5 mg, and identified as 3c; IR: 3500, 1200, 995 & 910cm^{-1}; NMR: 5.30 m (H-5), 3.90 s (overlapping signals of H-3 & H-8), 1.65 s br (H-11), 0.90 d (J=6.5 Hz, H-15); Mass spec.:m/z at 196 (M^+), 178, 163, 161 & 160.

Acetylation of 4a

A solution of 25 mg of 4a in 1 ml dry pyridine and 1 ml Ac_2O was heated at 100° for 8 hr. Usual work up procedure furnished the acetate 4b as an oil, yield 20 mg; IR: 1730, 1715, 1200, 1090, 1075, 1050, 960 & 850cm^{-1}; NMR: 4.05 s (overlapping signals of H-5 & H-8), 2.0 s (acetate methyl), 1.40 s (H-11), 1.00 s (9 protons, H-13, H-14 & H-15); Mass spec.:m/z 294 (M^+), 252, 234, 219 & 191.

$NaBH_4$ reduction of 4a

A solution of 25 mg of 4a in 2 ml MeOH was treated with 25 mg of $NaBH_4$ and the reaction mixture stirred at r.t. for 2 hr. It was diluted with H_2O, acidified with dil. AcOH and extracted with CH_2Cl_2 (5x100 ml). The washed and dried extract was evaporated and the crude material purified by PLC (Bz:EtOAc, 4:1) to furnish 20 mg of 4c as an oil. IR: 3500, 1120, 1090, 1000, 960 & 900cm^{-1}; NMR: 4.15 t (J=3 Hz, H-3), 3.60 (overlapping signals of H-5 & H-8), 1.40 s (H-11), 100 (overlapping signals of H-13, H-14 & H-15); Mass spec.:m/z 254 (M^+), 236 (M^+-H_2O), 218 (M^+-2H_2O), 203 ($M^+-2H_2O-CH_3$) & 175 ($M^+-2H_2O-C_3H_7$).

Acetylation of 4c with Ac_2O/Py as usual provided 4d as an oil. IR: 3500, 1730, 1110, 1080, 1000, 940 & 900cm^{-1}; NMR: 4.90 t (J=3 Hz, H-3), 4.10 m (H-5), 3.60 m (H-8), 2.00 s (acetate methyl), 1.38 s (H-11), 1.00 s (H-13, H-14 & H-15); Mass spec.:m/z 296 (M^+), 254, 236, 218, 203 & 175.

Collins oxidation of 4c to 4a

A solution of 60 mg of 4c in 2 ml dry dichloromethane was treated with 150 mg of CrO_3/2 Py complex and the reaction mixture was stirred at r.t. for 2 hr. The reaction was quenched with 1 ml methanol and after dilution with water was extracted with $CHCl_3$ (4x100 ml). The washed and dried extract was evaporated and the residue purified on TLC (Bz:EtOAc, 4:1) to furnish 20 mg of 4a as an oil.

Application of Horeau's method to 1a

A solution of 0.350g of α-phenylbutyric anhydride and 40 mg of 1a in 2 ml of dry pyridine was kept at r.t. for 64 hr. Excess anhydride was destroyed by addition of 10 ml of water and allowing the solution to stand for 12 hr. The reaction mixture was extracted with ether which was washed with water and 5% $NaHCO_3$ solution. The combined aqueous layers were washed with chloroform, acidified with $1N H_2SO_4$ and extracted with chloroform (3x100 ml). The washed and dried chloroform extract was evaporated, the residue 320 mg was pure α-phenylbutyric acid, $[\alpha]_D$ + 0.50° (c, 0.25, CCl_4) which corresponded to an optical yield of 14%.

Preparation of 1f

To a solution of 50 mg of 1a in 3 ml of pyridine, 200 mg of p-bromobensoyl chloride was added. After 72 hours the reaction mixture was diluted with 300 ml of CH_2Cl_2, washed with saturated $NaHCO_3$ solution and water respectively. The dried solution was evaporated; pyridine was removed by distilling with toluene in vaccuo. Purification of the crude by preparative TLC (Pet ether:EtOAc, 10:1) furnished 25 mg of 1f. M.p. 140°C. Single crystals were prepared from methanol solution used for X-ray crystallography. NMR: 7.5-8.00 overlapping signals for aromatic protons, 6.65 m (H-5), 5.15 m (H-8), 1.80 m (H-11), 1.10 overlapping signals of (H-13, 14, 15); MS:m/z at 419, 417(M^+), 218, 203.

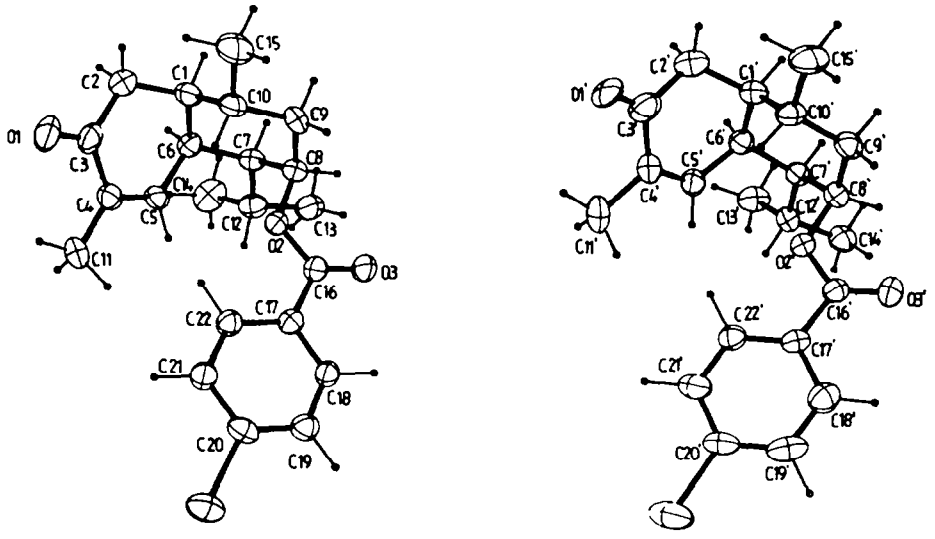


Fig. 1. A perspective view of two molecules present in the asymmetric unit cell with the atom numbering. Non-H atom ellipsoids were drawn at 25% probability level [19]. H-atom spheres are on arbitrary scale.

Acknowledgement :

We are grateful to our Director Dr J N Baruah for providing necessary facilities for this work; to Mr P R Bhattacharyya and Mr P Dutta for recording the antifeedant properties. One of us (MJB) thanks CSIR, India for a fellowship.

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