PART I

CHEMICAL EXAMINATION OF

*SAUSSOUREA AFFINIS*
RESULTS AND DISCUSSION

Saussurea affinis Spreng is a glabrous annual with thick stem, lyrate pinnatifid leaves and pink flowers, widely distributed in the North-eastern region of India such as Assam, Arunachal Pradesh and Mizoram. It is also found in some parts of Bangladesh, Nepal, Burma, China and Japan. The extract of the root is one of the ingredients used in medicines for the diseases of women.

Chemical examination of Saussurea affinis collected from the Itanagar area of Arunachal Pradesh led to the isolation of seven sesquiterpene lactones which are named here as (A), (B), (C), (D), (E), (F) and (G) in the order of their polarity and for the purpose of discussion that follows.

The least polar compound (A) was obtained as a gum. It exhibited spectral absorption typical of an \( \alpha \)-methylene-\( \gamma \)-lactone (i.r. band at 1760 cm\(^{-1}\)) of the type represented by the partial formula (X) because of the presence in the n.m.r. spectrum of the characteristic signals of \( H_a \) and \( H_b \) at 5.55 and 6.18 ppm.
The n.m.r. spectrum further revealed the presence of four narrowly split doublets near 4.90 and 5.40 ppm suggesting the presence of two exomethylene groups in compound (A). Two narrowly split triplets at 5.60 and 6.14 ppm were indicative of the presence of another methylene group \( \beta \) — to the ketone or ester grouping. A doublet of doublet of triplets at 4.51 ppm suggested the presence of a hydroxyl group in it which underwent paramagnetic shift to 5.54 ppm on acetylation. A three proton broad singlet at 1.96 ppm was indicative of the presence of a methyl on the double bond. A doublet of
doublet of doublets at 5.02 ppm was indicative of the presence of the ester group in compound (A). In the mass spectrum, the molecular ion peak at m/z 330 and a strong peak at m/z 69 suggested the presence of a four carbon ester side chain which was also supported by its i.r. spectrum (absorption band at 1715 cm\(^{-1}\)). It was identified as methacrylate ester side chain on the basis of n.m.r. spectrum as discussed earlier.

The above spectral data was essentially identical to that reported for aquerin B\(^4\)-\(^8\) and therefore structure (1a) was assigned to compound (A) and (1b) to its acetate.

\[
\begin{align*}
(1a) \quad & R = H \\
(1b) \quad & R = AC
\end{align*}
\]
Compound (B) was also obtained as a gum and in the mass spectrum the molecular ion peak at m/z 332 and a strong peak at m/z 71 suggested that compound (B) is an analogue of compound (A) where methacrylic side chain is replaced by isobutyryl ester side chain. Its n.m.r. spectrum was essentially identical with that of compound (A) except for the substitution of the signals of the methacryl for those of the isobutyryl ester side chain. Therefore compound (B) was identified as aquerin \( \text{A}^7 \text{(2a)} \). Acetylation of (2a) gave the monoacetate (2b).

\[
\begin{align*}
\text{(2a)} & \quad R = H \\
\text{(2b)} & \quad R = \text{Ac}
\end{align*}
\]
Compound (C) was also obtained as a gum. In the n.m.r. spectrum there were two broad singlets at 4.76 and 5.06 ppm each integrating to one proton, a triplet at 3.93 ppm with J=9 Hz, a doublet of triplet at 3.75 ppm with J=6 and 9Hz and two doublets with J=7Hz at 1.44 and 1.24 ppm each integrating to three protons. On the basis of above data structure (3a) was assigned to compound (C) and thus identified as isoamberboin. The mass spectrum exhibited molecular ion peak at m/z 264 in full agreement with the assigned structure (3a).

Acetylation of (3a) with acetic anhydride and pyridine furnished the monoacetate, jurmolide (3b) in whose n.m.r. spectrum H-8 underwent the expected paramagnetic shift to 4.93 ppm and the rest of the signals were essentially the same as that in isoamberboin (3a) besides the appearance of a acetate methyl at 2.12 ppm.

When the n.m.r. spectrum of compound (3b) was recorded in C₆D₆, C-11 methyl group underwent diamagnetic shift indicating it to be pseudoequatorial and therefore α.  

\[
(3a) \ R = \text{H} \\
(3b) \ R = \text{Ac}
\]
Compound (D) obtained as a gum was identified as cynaropicrin\textsuperscript{15, 16} (4a) by direct comparison (t.l.c., i.r., n.m.r. and m.s.) with its authentic sample. Acetylation of (4a) gave the acetate\textsuperscript{16} (4b).

\[
\begin{align*}
(4a) & \quad R = H \\
(4b) & \quad R = Ac
\end{align*}
\]

Compound (E) mp 135-137\textdegree was identified as 11\textbeta-H-11, 13-dihydrodesacylcynaropicrin\textsuperscript{16} (5a) by direct comparison (t.l.c., i.r., n.m.r. and m.s.) with its authentic sample\textsuperscript{16}. Acetylation of (5a) gave the acetate (5b).

\[
\begin{align*}
(5a) & \quad R = H \\
(5b) & \quad R = Ac
\end{align*}
\]
Compound (F) was obtained as a gum and analysed for C_{15}H_{22}O_6 in the high resolution mass spectrum. In the i.r. spectrum it exhibited absorption bands at 3500 and 1770 cm\(^{-1}\) indicating the presence of a hydroxyl group and a lactone moiety in it. The n.m.r. spectrum displayed signals at 5.01 and 5.07 ppm - two broad singlets each integrating to one proton, a triplet with J=7.5 Hz at 4.10 ppm for one proton, a double doublet with J=10.5 and 9.5 Hz at 4.21 ppm, a doublet of doublet of doublets with J=10, 8 and 5 Hz at 3.63 ppm. A broad singlet integrating to two protons at 3.87 ppm and a methyl doublet with J=7 Hz at 1.38 ppm. Acetylation of compound (F) furnished the triacetate in whose n.m.r. spectrum the signals at 4.10, 3.63 and 3.87 ppm in compound (F) underwent paramagnetic shift to 5.07, 4.88 and 4.36 ppm respectively suggesting that they represent the protons under hydroxyl groups. On the basis of above data and n.m.r. decoupling experiment on the triacetate, structure (6a) was assigned to compound (F) and (6b) to its triacetate.

In the mass spectrum the molecular ion peak at m/z 298 was in full accord with the assigned structure (6a). \(^{13}\)C n.m.r. spectrum (see experimental) of the acetate (6b) further confirmed the structure (6a) assigned to compound (F). The stereochemistry at C-3, C-6, C-7, C-8 and C-11 was identical
with those of its congeners (1a, 2a, 4a and 5a) but there remained the question of the stereochemistry at C-4 which was deduced as follows. Recording the n.m.r. spectrum of the acetate (6b) in the presence of trichloroacetylisocyanate resulted in the paramagnetic shift of H-3 and H-5 (see experimental) indicating that they are cis to the hydroxyl at C-4. Therefore, α-stereochemistry was assigned to the hydroxyl at C-4.

\[\text{(6a) } R = H \]
\[\text{(6b) } R = Ac\]
Compound (G) was obtained as a gum and was the most polar compound in this series. The i.r. spectrum showed strong absorption in the hydroxyl region viz. $3500\text{cm}^{-1}$ and a band at $1770\text{cm}^{-1}$ confirmed the presence of lactone moiety in it. Compound (G) on exposure to acetic anhydride and pyridine furnished the pentaacetate and on reaction with chlorotrimethylsilane and hexamethyl-disilazane furnished the pentatrimethylsilyl ether suggesting the presence of a hexose moiety in it. It was therefore decided to remove the hexose moiety in compound (G). Standard methods of hydrolysis glycosides led to extensive decomposition. However, reaction of compound (G) with sodium iodide in acetic acid, iodine on alumina in dimethylformamide and iodine in acetic acid led to the isolation of the aglycone in 30 to 40% yield in each case. These methods of cleaving the glycoside was therefore extended to solasonine (8a) and shanzhislde methyl ester (9a) and the results obtained are described in table 1. The reaction is based on the principle that the hydrogen iodide generated in situ is more reactive than the laboratory reagent. The reaction was reasonably clean and in all the three procedures no side products were observed. When the glycoside (compound (G)), solasonine (8a) and shanzhislde methyl ester (9a) were pretreated with sodium metaperiodate,
the reactions were quick and required milder reaction conditions and led to the increased yield of the aglycone. The aglycone obtained from compound (G) was identified as 11βH-11,13-dihydrodesacylcynaropicrin (5a) by direct comparison with its authentic sample.16

<table>
<thead>
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<th>TABLE 1</th>
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<td>Glycoside</td>
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<td>11βH-11,13-dihydrodesacylcynaropicrin 8β-D-glucoside (7a)</td>
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<td>solasonine (8a)</td>
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<td>shanzhiside methyl ester (9a)</td>
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<tr>
<td>11βH-11,13-dihydrodesacylcynaropicrin 8β-D-glucoside pretreated with NaI04</td>
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<td>solasonine pretreated with NaI04</td>
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<td>Glycoside</td>
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<td>shanzhiside methyl ester*</td>
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<td>11βH-11,13-dihydrodesacylcynaropicrin 8-β-D-glucoside (7b)</td>
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<td>solasonine (8a)</td>
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<td>shanzhiside methyl ester (9a)</td>
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<td>11βH-11,13-dihydrodesacylcynaropicrin 8-β-D-glucoside pretreated with NaI&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>shanzhiside methyl ester pretreated with NaI&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>solasonine (8a)</td>
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<td>Glycoside</td>
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<td>11βH-11, 13-dihydrodesacylcynaropicrin 8-β-D-glucoside pretreated with NaIO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>solasonine pretreated with NaIO&lt;sub&gt;4&lt;/sub&gt;</td>
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Enzymatic hydrolysis of compound (G) with β-D-glucosidase furnished compound 11β-11,13-dihydrodes-acylcynaropicrin (5a) and glucose identified by direct comparison with its authentic sample. Therefore, structure (7a) was assigned to compound (G) and (7c) and (7b) to its acetate and TMS ether.

(7a) \( R = H \), \( R' = \)

(7b) \( R = \text{TMS} \), \( R' = \)

(7c) \( R = \text{Ac} \), \( R' = \)
(7a) $R=H$, $R'=\beta$-glucose

(5a) $R, R' = H$

(9a) $R = \beta$-glucose

(9b) $R = H$

(8a) $R = \text{glucose-galactose-rhamnose}$

(8b) $R = H$
The n.m.r. spectrum of the acetate (7c) displayed the presence of two broad singlets at 5.42 and 5.29 ppm assignable to exomethylene protons at C-15, a broad singlet at 5.05 ppm integrating to two protons was assigned to H-14, a broad triplet at 5.53 ppm with J=7Hz was assigned to H-3, a double doublet with J=10 and 9Hz at 3.98 ppm was assigned to H-6, H-8 appeared as a doublet of doublet of doublets with J=10, 8 and 5Hz at 3.66 ppm, H-13 appeared as a doublet at 1.31 ppm, the protons due to the glucose moiety were at 4.74 ppm - a doublet with J=7.5Hz, 5.00 ppm - a triplet with J=8Hz, 5.21 ppm - a triplet with J=8Hz, 5.07 ppm - a triplet with J=9Hz, 3.76 ppm - a doublet of triplets with J=9 and 4.5Hz and a two proton multiplet at 4.19 ppm.

The glucose moiety was attached to the oxygen function on C-8 became apparent by comparison of the n.m.r. spectrum of the acetate (7c) with that of the TMS ether (7b), in the former H-3 signal appeared at a much lower field than the latter.

In the $^{13}$C n.m.r. spectrum of the acetate (7c) there were twelve doublets, five triplets, six quartets and eight singlets in full accord with the assigned structure.
GENERAL REMARKS

The following data apply to the experimental portions of all the parts unless otherwise stated.

Melting points were determined on a Kofler block and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 237B grating infra-red spectrophotometer as solution in chloroform or in film. Ultraviolet spectra were recorded on a Beckmann DU-2 spectrophotometer. Nuclear magnetic resonance (n.m.r.) spectra were recorded in deuterochloroform on either Varian T-60 or Bruker HX-230 instrument. Values are given in ppm (δ); unmarked signals are singlet, d=doublet, dd=double doublet, t=triplet, m=multiplet, br=broad singlet. Low resolution mass spectra were recorded on a MS-30 instrument and high resolution on MS-902.

Thin layer chromatography (t.l.c.) and preparative t.l.c. were performed on silica gel-G (BDH) and plates were activated at 110° for 1 hr. For column chromatography silica gel-G (60-120 mesh, BDH) was used. All solvents were distilled and dried before use.
EXPERIMENTAL

EXTRACTION OF SAUSUUREA AFFINIS SPRENG

Above ground parts of *Saussurea affinis* Spreng (2 kg) collected from the vicinity of Arunachal Pradesh, India (voucher on deposit in herbarium of RRL, Jorhat), were extracted with chloroform in a Soxhlet apparatus until the extract was colourless. After removal of solvent at reduced pressure, the residue (71 g) was dissolved in 300 ml methanol containing 30 ml water and left overnight at room temperature. It was then filtered and the filtrate washed with petroleum ether (60-80°, 6 x 300 ml), the methanol portion was concentrated at reduced pressure and the residue was thoroughly extracted with chloroform (8 x 200 ml). Evaporation of the washed and dried extract furnished 27 g of a gummy residue which was chromatographed over 500 g of silica gel in a column. 200 ml fractions being collected in the following order: fractions 1-13 (Bz), 14-113 (Bz-EtOAc, 9:1), 114-121 (Bz-EtOAc, 4:1), 122-179 (Bz-EtOAc, 2:1), 180-193 (Bz-EtOAc, 1:1), 194-249 (EtOAc), 250-269 (EtOAc-MeOH, 19:1), 270-275 (EtOAc-MeOH, 9:1), 276-280 (EtOAc-MeOH, 4:1) and 281-284 (EtOAc-MeOH, 2:1).

Fractions 58-74 (0.4 g) which exhibited two major spots on t.l.c. were combined and purified by preparative t.l.c. (Bz-EtOAc, 7:1, 4 developments). The faster moving
band yielded 54 mg of aguerin B (1a) as a gum; I.R.
bands at 3500, 1760, 1715, 1630, 1200, 1000 and 920 cm⁻¹;
the n.m.r. signals were at 4.51 (ddt, J=7.5, 1.5 Hz, H-3),
4.22 (dd, J=10.5, 9 Hz, H-6), 3.13 (ddt, J=9.5, 9, 3.5 Hz,
H-7), 5.02 (ddd, J=14.7, 10, 2.7 Hz, H-8), 5.55 (d, J=3.5 Hz,
H-13a), 6.18 (d, J=3 Hz, H-13b), 4.94 (d, J=2.1 Hz, H-14a),
5.10 (d, J=2.1 Hz, H-14b), 5.33 (d, J=1.5 Hz, H-15a), 5.47
(d, J=1.5 Hz, H-15b), 6.14 (t, J=1.2 Hz, H-18a), 5.60 (t,
J=1.2 Hz, H-18b) and 1.96 (H-19); mass spectrum gave the
molecular ion peak at m/z 330 (M⁺), and the other major
peaks were at m/z 244, 226, 197 and 69.

The slower moving band gave 45 mg of aguerin A (2a),
I.R. bands at 3500, 1760, 1720, 1630, 1200, 1035 and 915 cm⁻¹;
n.m.r. signals at 6.22 and 5.62 (d, J=3 Hz, H-13), 5.12 and
4.93 (d, J=2 Hz, H-14), 5.50 and 5.39 (d, J=2 Hz, H-15) and
1.20 (-CH(Me)₂); mass spectrum exhibited peaks at m/z
332 (M⁺), 314, 226, 244, 226, 197 and 71.

Fractions 81-94 (120 mg), which exhibited one major
spot, were combined and purified by preparative t.l.c.
(Bz-EtOAc, 2:1) to yield 30 mg of impure (by n.m.r. criteria)
isoamberboin (3a) as a gum. I.R. bands were at 1760, 1725,
1640, 1100 and 950 cm⁻¹; the n.m.r. (270 MHz) signals were
at 5.06 and 4.76 (br, H-14ab), 3.93 (t, J=9Hz, H-6), 3.75 (dt, J=6, 9Hz, H-8), 3.12 m (H-1), 2.82 (dd, J=13, 6Hz, H-9a), 2.5 (c, H-11, H-5), 2.25 (c, H-9b, H-2a,b), 2.05 (q, J=10Hz, H-7), 1.44 (d, J=7Hz, H-13) and 1.24 (d, J=7Hz, H-15); mass spectrum exhibited peaks at m/z 264 (M+), 246, 218 and 203.

Fractions 117-119 (1.5 g) showed one major spot on t.l.c. and was purified by preparative t.l.c. (Bz-EtOAc, 2:1) to yield 1.2 g cynaropicrin (4a) as a gum, identified by direct comparison (t.l.c., i.r., n.m.r. and m.s.) with an authentic sample.16

Fractions 120-151 were combined to give 480 mg of crude material which was purified by preparative t.l.c. (Bz-EtOAc, 2:1) to furnish 52 mg of (5a), mp 134° (lit16 mp 136-137°) identified by direct comparison (t.l.c., i.r., n.m.r. and m.s.) with an authentic sample.16

Fractions 210-224 (1.0 g) were combined and purified by preparative t.l.c. (CHCl3-MeOH, 22:3) to give 120 mg of saussureolide (6a) as a gum; i.r. (film) bands at 3500, 1770, 1050 cm⁻¹; mass spectrum exhibited peaks at m/z 298 (M+), 280, 267, 262, 249 and 231. [Calcd. for C15H22O6: Mw, 298.1416. Found: Mw(m.s.), 298.1419]. The n.m.r. (270 MHz, CDCl3 and 3 drops of DMSO-d6) signals were at
3.06 (br q, H-1), 2.19 (ddd, J=14, 10, 7.5Hz, H-2a),
1.81 (ddd, J=14, 10, 7.5Hz, H-2b), 4.10 (t, J=7.5Hz,
H-3), 2.26 (t, J=10.5Hz, H-5), 4.21 (dd, J=10.5, 9.5Hz,
H-6), 1.90 (q, J=10Hz, H-7), 3.63 (ddd, J=10, 8, 5Hz, H-8),
2.77 (dd, J=13, 5Hz, H-9a), 2.07 (dd, J=13, 8Hz, H-9b),
2.53 (dq, J=10, 7Hz, H-11), 1.38 (d, J=7Hz, H-13), 5.07
(br, H-14a), 5.01 (br, H-14b) and 3.87 (H-15).

Fractions 254-272 (500 mg) were combined and purified
by preparative t.l.c. (CHCl₃-MeOH, 4:1) to furnish 80 mg of
(7a) as a gum; i.r. bands at 3500, 1770 and 1050cm⁻¹. The
low resolution mass spectrum did not exhibit the molecular
ion.

Acetylation of (1a)

To a solution of 10 mg of (1a) in 1 ml pyridine was
added 2 ml acetic anhydride and left overnight at room
temperature. The mixture was poured into 100 ml water,
extracted with chloroform and dried over anhydrous sodium
sulphate. The washed and dried extract was concentrated
under reduced pressure, pyridine removed by co-distillation
with toluene and the residue was purified by preparative
t.l.c. (Bz-EtOAc, 9:1) to give 10 mg of (1b) as a gum;
i.r. bands at 1760, 1710, 1630, 1225, 1150 and 1000 cm\(^{-1}\); 
\(^1\)H n.m.r. signals were at 6.22 and 5.61 (d, J=3 Hz, H-13), 
5.15 and 4.96 (d, J=2 Hz, H-14), 5.42 (d, J=2 Hz, H-15), 
5.54 (t, J=8 Hz, H-3), 5.03 (ddd, J=14.7, 10, 2.7 Hz, H-8), 
1.97 (H-19) and 2.10 (OAc); mass spectrum exhibited peaks 
at m/z 372 (M\(^+\)), 330, 312, 244, 226, 197 and 69.

**Acetylation of (2a)**

A solution of 8 mg of (2a) was acetylated with 1 ml 
pyridine and 2 ml acetic anhydride overnight at room 
temperature. After usual work up it furnished 8 mg of (2b) 
as a gum whose i.r. bands appeared at 1760, 1725, 1630, 
1220, 1150 and 1000 cm\(^{-1}\); \(^1\)H n.m.r. signals were at 6.23 
and 5.62 (d, J=3 Hz, H-13), 5.62 and 5.40 (d, J=3 Hz, H-15), 
5.14 and 4.99 (d, J=2 Hz, H-14), 1.22 (\(^{-}\)CH\((\text{Me})_2\)) and 2.12 (OAc); 
mass spectrum exhibited peaks at m/z 374 (M\(^+\)), 332, 244, 226, 
197 and 71.
Preparation of jurmolide (3b)

A solution of 10 mg of (3a) in 1 ml pyridine and 2 ml acetic anhydride was kept overnight at room temperature. The reaction mixture was diluted with 100 ml water, extracted with chloroform and dried over anhyd. sodium sulphate. Removal of solvent under reduced pressure furnished a crude which on purification by preparative t.l.c. (Bz-EtOAc, 6:1) gave 10 mg of jurmolide (3b). The compound was pure but could not be induced to crystallize. It had i.r. bands at 1760, 1730 (broad), 1650, 1100 and 950 cm⁻¹; mass spectrum exhibited peaks at m/z 306 (M⁺), 264, 246, 218, and 203; ¹H n.m.r. (270 MHz, CDCl₃) signals at 5.15 and 4.82 (br, H-14a,b), 4.93 (dt, J=6, 9Hz, H-8), 4.01 (t, J=9Hz, H-6), 3.11 (m, H-1), 2.89 (dd, J=13, 6Hz, H-9a), 2.54 (t, J=9Hz, H-5), 2.4 (c, H-11, H2a), 2.24 (c, H-7, H-2b), 2.12 (OAc), 1.35 (d, J=7Hz, H-13) and 1.24 (d, J=7Hz, H-15); ¹H n.m.r. (C₆D₆) (decoupled to verify J₇,11) signals at 2.14 (dt, J=3, 9Hz, H-1), 2.06 (dd, J=18, 3Hz, H-2a), 1.9 (dd, J=18, 9Hz, H-2b), 1.81 (m, H-4), 1.34 (m, H-5), 2.90 (t, J=9.5 Hz, H-6), 1.74 (q, J=10Hz, H-7), 4.48 (dt, J=5, 10Hz, H-8), 2.54 (dd, J=12, 5Hz, H-9a), 1.63 (dd, J=12, 10Hz, H-9b), 1.94 (m, H-11), 1.20 (d, J=7Hz, H-13), 4.63 and 4.34 (br, H-14a,b), 1.20 (d, J=7Hz, H-15), and 1.62 (OAc)
value of \( J_{7,11} \) (10 Hz) and the solvent shift of the 
H-13 signal (\( \delta_{\text{CDCl}_3} - \delta_{\text{C}_6\text{H}_6 + 0.15} \)) indicated that the 
C-11 methyl group was pseudoequatorial and \( \alpha \).

**Acetylation of (6a)**

To a solution of 50 mg of (6a) in 1 ml pyridine 
was added 2 ml acetic anhydride and left overnight at 
room temperature. The reaction mixture after usual work 
up and purification by preparative t.l.c. (Bz-EtOAc, 2:1) 
gave 50 mg of (6b) as a gum; \(^{1}\text{H n.m.r.} \) (270 MHz) signals 
were at 3.21 (br q, H-1), 2.32 (ddd, J=14, 10, 7.5 Hz, 
H-2a), 1.78 (ddd, J=14, 10, 7.5 Hz, H-2b), 5.07 (t, J=7.5 Hz, 
H-3), 2.43 (t, J=10.5 Hz, H-5), 4.23 (dd, J=10.5, 9.5 Hz, H-6), 
2.17 (q, J=10 Hz, H-7), 4.88 (ddd, J=10, 8, 5 Hz, H-8), 2.72 
(dd, J=13.5 Hz, H-9a), 2.13 (dd, J=13, 8 Hz, H-9b), 2.46 
(dq, J=10.7 Hz, H-11), 1.23 (d, J=7 Hz, H-13), 5.11 (br, 
H-14), 5.09 (br, H-14b), 4.36 (H-15), 2.09, 2.08 and 
2.06 (OAc). The \(^{1}\text{H n.m.r.} \) (270 MHz) signals of (6b) with 
a drop of trichloroacetylisocynate were at 3.30 (br q, 
H-1), 2.47 (m, H-2a), 1.91 (ddd, J=14, 10, 7.5 Hz, H-2b), 
5.81 (t, J=7.5 Hz, H-3), 3.44 (t, J=10.5 Hz, H-5), 4.33 
(dd, J=10.5, 9.5 Hz, H-6), 2.23 (m, H-7), 4.94 (ddd, J=10, 
8, 5 Hz, H-8), 2.68 (dd, J=13.5 Hz, H-9a), 2.23 (m, H-9b),
2.47 (m, H-11), 1.30 (d, J=7Hz, H-13), 5.17 (br, H-14a),
5.09 (br, H-14b), 4.72 (H-15), 2.12, 2.11, 2.06 (OAc)
and 8.45 (NjH). It had i.r. bands at 3550, 1775, 1725,
1630, 1250 and 1025cm⁻¹. ¹³C n.m.r spectrum is listed
in table 2.

Acetylation of (7a)

To a solution of 40 mg of (7a) in 1 ml pyridine
was added 2 ml acetic anhydride and left overnight at
room temperature. The reaction mixture after usual work up
and purification by preparative t.l.c. (Bz-EtOAc, 2:1)
yielded 50 mg of (7c) as a gum. It had i.r. bands at
1775, 1750-1720 (broad band); ¹H n.m.r. (270 MHz) signals
at 2.98 (br q, J=9Hz, H-1), 2.43 (m, H-2a), 1.77 (ddd,
J=12,8,7Hz, H-2b), 5.53 (br t, J=7Hz, H-3), 2.80 (br t,
J=7Hz, H-5), 3.98 (ddd, J=10,9Hz, H-6), 2.20 (q, J=10Hz,
H-7), 3.66 (ddd, J=10,8,5Hz, H-8), 2.78 (dd, J=14,5Hz,
H-9a), 2.43 (m, H-9b), 2.43 (m, H-11), 1.31 (d, J=7Hz,
H-13), 5.05 (br, H-14), 5.42 (br, H-15a), 5.29 (br, H-15b),
2.09, 2.06, 2.03, 2.03 and 2.00 (OAc), 4.74 (d, J=7.5Hz,
H-1'), 5.00 (t, J=8Hz, H-2'), 5.21 (t, J=8Hz, H-3'), 5.07
(t, J=9Hz, H-4'), 3.76 (dt, J=9,4.5Hz, H-5'), and 4.19
(H-6'); ¹³C n.m.r. spectrum is listed in table 2.
TABLE 2

$^{13}$C n.m.r. spectra* of (6b) and (7c)

<table>
<thead>
<tr>
<th>Carbon</th>
<th>(6b)</th>
<th>(7c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 1</td>
<td>41.46$\dagger$</td>
<td>44.37$\dagger$</td>
</tr>
<tr>
<td>C - 2</td>
<td>32.79$\dagger$</td>
<td>36.34$\dagger$</td>
</tr>
<tr>
<td>C - 3</td>
<td>78.24$\dagger$</td>
<td>74.67$\dagger$</td>
</tr>
<tr>
<td>C - 4</td>
<td>81.39</td>
<td>147.89</td>
</tr>
<tr>
<td>C - 5</td>
<td>55.00$\dagger$</td>
<td>50.91$\dagger$</td>
</tr>
<tr>
<td>C - 6</td>
<td>76.90d</td>
<td>78.92d</td>
</tr>
<tr>
<td>C - 7</td>
<td>53.66$\dagger$</td>
<td>53.93$\dagger$</td>
</tr>
<tr>
<td>C - 8</td>
<td>75.43d</td>
<td>85.10$\dagger$</td>
</tr>
<tr>
<td>C - 9</td>
<td>42.03t</td>
<td>42.55t</td>
</tr>
<tr>
<td>C - 10</td>
<td>140.49</td>
<td>142.56</td>
</tr>
<tr>
<td>C - 11</td>
<td>40.46d</td>
<td>41.46d</td>
</tr>
<tr>
<td>C - 12</td>
<td>176.85</td>
<td>177.94</td>
</tr>
<tr>
<td>C - 13</td>
<td>15.71q</td>
<td>16.12q</td>
</tr>
<tr>
<td>C - 14</td>
<td>117.19t</td>
<td>116.81t</td>
</tr>
<tr>
<td>C - 15</td>
<td>64.13t</td>
<td>114.33t</td>
</tr>
<tr>
<td>Carbon</td>
<td>(6b)</td>
<td>(7c)</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>C - 1'</td>
<td>101.28d</td>
<td></td>
</tr>
<tr>
<td>C - 2'</td>
<td>71.94d</td>
<td></td>
</tr>
<tr>
<td>C - 3'</td>
<td>73.08d</td>
<td></td>
</tr>
<tr>
<td>C - 4'</td>
<td>68.46d</td>
<td></td>
</tr>
<tr>
<td>C - 5'</td>
<td>72.05d</td>
<td></td>
</tr>
<tr>
<td>C - 6'</td>
<td>62.19t</td>
<td></td>
</tr>
</tbody>
</table>

*Run in CDCl₃ at 67.9 MHz on Bruker HX-270 instrument. Values are in parts per million. Unmarked signals are singlets.

†Assignment by selective spin decoupling.
**Preparation of (7b)**

A solution of 10 mg of (7a) in 1 ml hexamethyl-disilazane and 0.5 ml chlorotrimethylsilane was kept overnight at room temperature. The excess reagents were removed under vacuum and the pentatrimethylsilyl-ether (7b) was obtained as a gum in quantitative yield which needed no purification. It had \(^1\)H n.m.r. (270 MHz) signals at 2.82 (br q, J=9Hz, H-1), 2.08 (ddd, J=12,8, 7Hz, H-2a), 1.66 (ddd, J=12,8,7Hz, H-2b), 4.43 (br t, J=7Hz, H-3), 2.73 (br t, J=7Hz, H-5), 4.01 (t, J=10Hz, H-6), 2.20 (q, J=10Hz, H-7), 3.71 (ddd, J=10,8,5Hz, H-8), 2.66 (dd, J=14,5Hz, H-9a), 2.37 (dd, J=14,8Hz, H-9b), 2.48 (dq, J=10,5,7Hz, H-11), 1.43 (d, J=7Hz, H-13), 5.01 (br, H-14a), 4.98 (br, H-14b), 5.36 (br, H-15a), 5.36 (br, H-15b), 4.34 (d, J=7.5Hz, H-1'), 3.23 (t, J=8Hz, H-2'), 3.37 (t, J=8Hz, H-3'), 3.44 (t, J=9Hz, H-4'), 3.21 (dt, J=4.5,9Hz, H-5') and 3.71 (H-6').
Enzymatic hydrolysis of (7a)

A mixture of 28 mg (7a) in 1.5 ml water and 18 mg \(\beta-D\)-glucosidase was stirred for 12 hr at room temperature during which time t.l.c. indicated disappearance of the starting material. The mixture was diluted with 30 ml water and extracted with ethyl acetate. Evaporation of the washed and dried extract under reduced pressure gave after purification by preparative t.l.c. (Bz-EtOAc, 2:1) 15 mg of (5a) as a gum, identical (t.l.c., i.r., n.m.r. and m.s.) with an authentic sample.\(^{16}\)

The aqueous portion left after extraction with ethyl acetate was thoroughly extracted with n-butanol. Evaporation of the washed and dried extract under reduced pressure gave a crude which on purification by preparative t.l.c. (CHCl\(_3\)-MeOH, 3:2) yielded 6 mg of glucose.
Reaction of glycosides with sodium iodide in acetic acid

General procedure

A solution of glycoside (30 mg) in acetic acid (2 ml) was treated with sodium iodide (200 mg) and the reaction mixture subjected to the conditions indicated in table 1. The reaction mixture was worked up as follows. It was diluted with 50 ml water and extracted thoroughly with ethyl acetate. The extract was washed with 2N sodium thiosulphate, then with water and finally dried over anhydrous sodium sulphate. It was then evaporated under reduced pressure and traces of acetic acid was removed by co-distillation with toluene. The residue was purified by preparative t.l.c. (CHCl₃ - MeOH, 5:1) to furnish the corresponding aglycone as a gum. The unreacted glycoside was recovered as a gum from the aqueous portion by extraction with n-butanol and purified by preparative t.l.c. (CHCl₃ - MeOH, 3:1) on silica gel.

Reaction of glycosides with iodine on alumina

General procedure

A solution of glycoside (30 mg) in dimethylformamide (2 ml) was treated with iodine (15 mg) and alumina (300 mg)
and the reaction mixture was subjected to the conditions indicated in table 1. Reaction mixture was worked up as described above to furnish the aglycone and the unreacted glycoside.

**Reaction of glycosides with iodine in acetic acid**

General procedure

A solution of glycoside (30 mg) in acetic acid (2 ml) was treated with iodine (15 mg) and the reaction mixture was subjected to the conditions stated in table 1. Reaction mixture was worked up as described above to furnish the aglycone and the unreacted glycoside.

**Reaction of glycosides with sodium metaperiodate**

A solution of glycoside (30 mg) in methanol (2 ml) was treated with sodium metaperiodate (100 mg) in water (1 ml) and the reaction mixture left aside at room temperature in the dark overnight. This reaction mixture was termed as glycoside pretreated with sodium metaperiodate.
REFERENCES

1. 'The Wealth of India', Raw materials, 1972, IX, 244 (Published by CSIR, India).


