STRUCTURE OF A NEW TRITERPENE GLYCOSIDE FROM *CENTROSEMA BRACTEOSUM**

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RESUMO

Um novo Triperpeno 3-0-[α -L-Rhamnopiranosil (1→2)-(β-D-xy-lopiranosil 16 β, 23(R):16α, 30α - diepoxi-20(2)-hidroxidammar-24-eno(1) foi isolado a partir das raizes de *centrosema bracteosum*. A estrutura deste glicosídeo triterpenoidico foi elucidada por análise espectroscópica do produto natural (1) e seu derivado peracetil (1a), incluindo experimentos de 2D RMN.

Palavras chave: *Centrosema bracteosum*, Leguminosae-Faboideae, Glicosídeo Triterpenoidico, Dados espectrais.

ABSTRACT

A new triterpene 3-O-[α-L-Rhamnopyranosyl (1→2)-(β-D-xy-lopyranosyl 16β,23 (R): 16α,30α-diepoxy-20(S)-hydroxydammar-24-ene (1) was isolated from roots of *Centrosema bracteosum*. The structure of this triterpenoid glycoside was elucidated by spectroscopic analysis of the natural product (1) and its peracetyl derivative (1a), including 2D NMR experiments.

Keywords: *Centrosema bracteosum*; Leguminosae-Faboideae; triterpene glycoside; spectral data.

INTRODUCTION

Centrosema bracteosum is a medicinal plant used as stomatic in popular medicine and was submitted to biological studiesl. These additional information contributed to the chemical investigation of a specimen of this species, as part of our continuing research with brazilian plants. We have isolated from the ethanolic roots extract a new pentacyclic triterpenoid saponin **1**. The structure of this triterpene glycoside was deter mined by spectrometric data analysis of **1** and its peracetyl derivative (**1a**).

RESULTS AND DISCUSSION

The IR spectrum of triterpene glycoside **1** revealed the absence of absorption due to ester or ketone carbonyl group and showed a strong absorption band (3400cm^{-1}) for hydroxyl functions. The molecular formula C60H88O23

for the peracetyl derivative **1a** was determined on the basis of its molecular ion at *m/z* 1176, deduced by fast atom bombardment mass spectrum [FABMS: $(SCH_2CH_2OH)_2$ matrix reference, negative ion mode] in combination with the ^1H and ^{13}C NMR (PND = Proton-Noise Decoupled and DEPT = Distortionless Enhancement by Polarization Transfer) spectra (Tables 1 and 2). The ^1H and ^{13}C NMR spectra showed the presence of three anomeric carbons [δ_H ($CDCl_3$) 4.46 (*d*, J=5.9 Hz, H-1'), 4.63 (*d*, J=6.6 Hz, H-1") and 4.89 (*br s*, H-1'"); δ_C 103.47 (*d*, C-1'), 100.94 (*d*, C-1") and 97.94 (*d*, C-1'")], signals for five tertiary methyl protons attached to sp3 carbons [dH ($CDCl_3$) 1.14 (*s*), 1.06 (*s*), 0.94 (*s*), 0.80 (*s*) and 0.77 (*s*)] and two to sp^2 [δ_H 1.67 (*br s*) and 1.63 (*br s*)], along with one bound to a secondary sp3 carbon (δ_H 1.15 (*d*, J=5.8 Hz) corresponding to the methyl group of the rhamnose, and seven acetyl functions [δ_H ($CDCl_3$) 2.08 (*s*), 2.06 (*s*), 2.05 (*s*), 2.01 (*s*), 1.99 (*s*), 1.97 (*s*) and 1.94 (*s*)] (Tables 1 and 2). The mass spectrum obtained for electron impact (EIMS) of the peracetyl derivative **1a** did not show the peak corresponding to molecular ion ($M^{+\cdot}$ 1176) but revealed peaks at *m/z* 489 (6%), 437 (6%), 273 (99%), 213 (24%) and 153 (100%) which were attributed to the fragment ions **2-6**, respectively. The fast atom bombardment mass spectrum [FABMS: $SCH_2CH_2OH)_2$ matrix reference, negative ion mode] of the natural product **1** exhibited a molecular ion peak at *m/z* 882 base peak), consistent with a molecular formula $C_{46}H_{74}O_{16}$, and peaks at *m/z* 736 (15%), 266 (22%) and 185 (41%), which were correlated with fragment ions **7**, **7a** and **8**, corresponding to the loss of terminal 6-dehydroxylhexose (**7**) and the production of the ions pentose-pentose disaccharide (**7a**) and hemiacetal (**8**). These sugars were characterized as L-rhamnose and D-xylose (two molecules) with the aid of ^1H and ^{13}C NMR spectra (Table 1) including 2D-shift-correlated $^1Hx^1H$-COSY (homonuclear) and $^1Hx^{13}C$-COSY-$^1J_{CH}$ (heteronuclear, modu lated to $^1J_{CH}$)[2]. In fact upon hydrolysis, **1** furnished L-rhamnose and D-xylose (1:2) which were identified through thin layer chromatography (TLC) by comparison with authentic samples. Thus, the presence of the seven acetyl groups observed during the ^1H NMR spectrum analysis of **1a** represents the esterification of the seven hydroxyl groups of the sugar moiety and, consequently, the aglycone skeleton does not sustain hydroxy group for acetylation in the conditions utilized. From the above arguments the sugar moiety was classified as a trisaccharide containing one L-rhamnose molecule and two D xylose molecules. The ^1H and ^{13}C NMR sugar signals (Table 1) of peracetyl derivative **1a** were in agreement with a terminal L-rhamnose unit linked at C-2 of an inner D-xylose unit and the inner D-xylose linked at C-2 of another inner D-xylose unit. As shown in Table 1, the chemical shifts of the H-2' [δ ($CDCl_3$) 3.8 (*dd*, J=5.9 and 8.4 Hz)], H-2" [δ(CDCl3) 3.57 (*dd*, J=6.6 and 8.6 Hz)], along with C-2' [δ 74.51 (*d*)] and C-2" [δ 75.85 (*d*)], indicated that the acetylation did not affect these signals. Acetylation deshields all the CH α to acetates, appearing in the 4.5 - 5.5 ppm region, and leaves unaltered all the α protons to branching points (α to ether functions). This sequencing of the sugar chain on the peracetylated **1a** was confirmed by ^1H x ^1H-COSY spectrum, which clearly revealed the interactions of the H-1' [δ(CDCl3) 4.46 (δ, J=5.9 Hz)] with H-2' [δ (CDCl3) 3.80] and H-1" [d (CDCl3) 4.63 (δ, J=6.6 Hz)] with H-2", [d (CDCl3) 3.57] and the NOEs observed for the H-I" (6.4%) and H-1'" (11%) upon irradiation of the H-2' and H-2", respectively. All ^1H and ^{13}C NMR signals of the sugar moiety were as signed as listed in Table 1 by the DEPT experiment, ^1H x ^1H- COSY and ^1H x ^{13}C-COSY spectra. The chemical shifts of the four methylenic protons (δ 3.3-42) and two carbon atoms C-5' and C-5" (δ 62.01 and 61.48) were used to established the presence to two molecules of pentose D-xylose as D-xylopyranosyl units. Additional confirmation of terminal system α-L-rhamnopyranosyl (1→2)-xylopyranosyl was obtained by comparison of the ^{13}C NMR chemical shifts of **1** with the values described for the model compound **13**[3]. Based on the evidences mentioned above, the sugar moiety was defined as -O-α-L-rhamnopyranosyl (1→2)-b-D-xylopyranosyl (1→2)-β-D-xylopyranoside peracetylated, corresponding to the partial molecular formula $C_{30}H_{41}O_{20}$.

The remaining ^{13}C NMR signal represents the aglycone moiety (Table 2). The molecular formula $C_{30}H_{47}O_3$ for this aglycone was also deduced by difference between the molecular formula of **1a** ($C_{60}H_{88}O_{23}$) and the sugar moiety ($C_{30}H_{41}O_{20}$), which analysed in combination with the ^1H and ^{13}C NMR and mass data allowed clearly to classify the aglycone moiety as triterpenoid. The presence of two methyl singlet (broad) signals attached to sp2 carbon atom (δ 1.67 and 1.63) in the ^1H NMR spectrum and only one double bond (trisubstituted) revealed by ^{13}C NMR spectrum [δ125.04 (*d*) and 135.28 (*s*)] pointed to the localization of this unsaturation between carbon atoms C-24 and C-25 and, consequently, allowed to classify the triterpene as tetracarbocyclic with side-chain carbons at C-17. The difference between the unsaturation number (seven) obtained of the partial molecular formula ($C_{30}H_{47}O_3$) and those corresponding to tetracarbocyclic system (four) and to double bond (one) indicated the existence of two additional rings. The ^{13}C NMR spectra showed also the presence of signals attributed to carbon atoms sustaining oxygen atoms at δ 109.48 (*s*), 89.69 (*d*), 69.23 (*s*), 68.60 (*d*), and 65.72 (*t*). The linkage between the trisaccharide and the aglycone was shown to be at C-3 by the ^{13}C absorption at δ 89.69 in comparison with the absorption at d 88.10 in the model compound **94**, containing the same aglicone (*vide infra*), demonstrating by the observed downfield field ($\Delta\delta$=10.79) that this carbon sustains an ether glycosidation (*e.g.* **10** and **11**, Table 2). This deduction was confirmed by an upfield shift of C-2 [δ 25.85 (*t*)] when compared with the signal at δ 27.40 of the model compound **10**[5], the chemical shift of the H-3 (δ 3.1-2.9) in the ^1H NMR spectrum and by biogenetic arguments because of the frequent presence of oxi group at C-3 of triterpenoids[6]. The location of a tertiary hydroxyl group at C-20 was derived from the signal at δ 69.23 (*s*, quaternary carbon) in the ^{13}C NMR and a methyl singlet signal at δ 1.14 in the ^1H NMR spectrum which was 2D-shift-correlated2 by the heteronuclear ^1H x ^{13}C-COSY-$^1J_{CH}$ spectrum with the carbon methyl signal at δ 29.84 (Table 2). The remaining two oxygen atoms were used to justify the presence of a ketal, an oxymethylene and oxymethine functions and two additional rings in the aglycone on the basis of the chemical shifts at δ 109.48 (*s*), 65.72 (*t*), and 68.60 (*d*), respectively, in the ^{13}C NMR and unsaturation number. The homonuclear coupling of the oxymethine H-23 (δ 4.60) with H-24 (δ 5.15) and with H-22 (d 1.6-1.4) were revealed by 2D-shift-correlated (^1H x ^1H-COSY) spectra. These data are consistent with an ether function 16,23-epoxy and ketal group was established with another ether group 16,30-epoxy, containing the oxymethylene as show in **1** and **1a**. Thus, the aglycone was defined as a triterpene with the basic skeleton of the dammarane type.

The data from ^1H and ^{13}C NMR uni (1D) and two-dimensionals (2D) spectra of the natural product **1**, recorded in pyridine-d_5 (Tables 1 and 2), together with comparison of the sugar moiety with the model saponin **13**[3] containing an analogous terminal system α-L-rhamnopyranosyl (1→2)-β-D-xylopyranosyl (Table 1) revealed themselves consistent with the deduction described above.

The assignment of an equatorial-position for the O-trisaccharide moiety at C-3 was deduced from the chemical shifts of carbons 1 to 5, 28 and 29 of **1a** (Table 2) when compared with the model compounds **9**[4], **10**, **12**[7] and **11**[5].

The chemical shifts [δ_C 29.84 (**Ia**); 30.27 (**1**)] of the methyl group at C-20 in the ^{13}C NMR spectra of **1** and **1a** suggested an equatorial configuration (and axial hydroxy, **1b**). The signal of a methyl group at an axial-position in a hexacyclic system appears with a smaller chemical shift than δ_C20 because of g-effects (*e.g.* in the Table 2). The assignment of an equatorial-orientation for the CH=CMe_2 group at C-23 was deduced from the coupling constant J=8.9 Hz observed in the signal of H-23 [δ_H 5.27 (*dd*, J=8.9, 6.0 Hz)] in the ^1H NMR spectrum of **1**. This value of J=8.9 Hz is only consistent with H-23 at an axial-position (**Ib**).

Based upon the above data, the structure of new triterpene saponin **1** was established as

3b-O-[α-L-rhamnopyranosyl (1→2)-β-D-xylopyranosyl (1→2)-β-D-xylopyranosyl]-16β,23(R): 16α,30α-diepoxy-20(S)-hydroxydammar-24-ene (**1**) and its peracetyl derivative as 3β-O-[α-L-2''',3''',4'''-tri-O-acetylrhamnopyranosyl (1→2)-b-D-3'',4''-di-O-acetylxylopyranosyl (1→2)-b-D-3,4-di-O-acetylxylopyranosyl]16β,23(R);16α,30α-diepoxy-20(S)-hydroxyldammar-24-ene (**1a**). All ^{13}C NMR signals were reasonably assigned as listed in Tables 1 and 2 through the chemical shifts, multiplicity deduced of DEPT, comparison with models (*e.g.* compounds **9-11**), application of the usual shift parameters and 2D NMR spectra (^1H x ^1H-COSY,^1H x ^{13}C-COSY). Furthermore, the stereochemistry of the aglycone (**1b**) of **1** and **1a** is in agreement with the prediction on the basis of a biogenetic route of the secondary metabolism for the biaproduction of dammarane skeleton6. The NOE difference experiments were used in the case of the sequencing of the sugar chain, which also indicated the preferred conformation to establish the adequate spatial proximity of H-2' to H-1'' and of H-2'' to H-1''', as shown in **1** and **1a**.

The biosynthesis of this new triterpene has not yet been investigated but it might involve a sequence of reactions enzymatically controlled as described in the literature6. Suggestion about the secondary biocyclization to produce the two heterocycle rings (16β,23-epoxy and 16α,30α-epoxy) include oxidation at C-16 (carbonyl or hydroxy groups), C-23 (hydroxyl or carbonyl groups) and C-30 (hydroxyl group), enzimatically induced, to formation of a precursor 16-oxo-23,30-dihydroxy or 16,23-dioxo-30-hydroxy. These precursors present the appropriate functional groups for the biocyclization and production of the two heterocycle rings involving two ethers and one ketal functions (Chart 1). The stereochemistries at chiral carbon atoms C-5, C-8, C-9, C-13, C-14, and C-17 are those antecipated by the biogenetic route of dammarane skeleton6. Since the configuration of the hydroxymethylene at C-14 is a, the attack of its hydroxy group on C-16 can only be carried out by the same side, consequently leading to the formation $16_a,30_a$-epoxy function. The glycosidation is a common process of secondary metabolism[6].

After having submitted this work for publication (January 5,1994) which returned to us with some corrections and suggestions from two referees, we received *J. Nat. Prod.* (1994), **57** (2) with a paper which reported the structure elucidation of three triterpenoid glycosides containing the same aglycone (jujubogenin) of **1**[8]. In that paper, the configuration at C-17 of the aglycone had the hydrogen atom with b orientation (and D/E-*trans* ring fusion) on the basis of a comparison with the literature: "the structure of a derivative has been confirmed by chemical evidence as well as by X-ray crystallography"[9,10]. The comparison of the NMR spectral data, mainly the ^{13}C NMR data, indicated that the aglycone of the three glycosides[8] is identical to the one presente in **1**, including H-23 and hydroxyl group at C-20 at axial position. After this comparison and additional evaluation of the papers[9,10] (cited in reference 10 as 6 and 7) we remain convinced of the D/E-*cis* ring fusion (**1b**), for which the structure defined by X-ray crystallography10 is more suitable. This configuration is also the one suggested by the biogenetic route of a dammarane skeleton[6] and by the smaller chemical shift of C-13 methine carbon [δ_C 36.85 (*d*)] of **1** (g-effect of hydroxyl groups at C-20) when compared with the model **10** [δ_C 42.3 (C-13)][7] (Table 2).

EXPERIMENTAL

General experimental procedures. Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. The ^1H (200 MHz) and ^{13}C (50.3 MHz) NMR spectra were obtained in C_5D_5N, $CDCl_3$ and C_6D_6 with TMS as an internal standard, employing a Bruker AC-200 spectrometer; standard Bruker pulse sequences were used to perform ^1H x ^1H-COSY and ^1H x ^{13}C-COSY; proton-noise decoupled and DEPT - ^{13}C NMR were utilized to recognize the number of attached protons for each carbon atom. IR spectra were recorded as KBr discs on a Perkin-Elmer spectrometer. Low resolution mass spectra were obtained on a Hewlett Packard - 5890/5988A GC/MS

instrument operating at 70 eV and FABMS on a Kratos MS-50 in the negative ion mode. Column chromatography was run with silica gel 60 (70-230 mesh, E. Merck, Darmstadt, Germany). Thin layer chromatography was performed on silica gel 60 F 254 (Merck).

Plant material. A specimen of *Centrosema bracteosum* Bentham, Leguminosae--Faboideae, was collected in Reserva Ecológica do Instituto Brasileiro de Geografia e Estatística, Brasília, DF, and identified by Drs. Ezequias Paula Heringer and Geraldo Ismael Rocha. A voucher specimen is deposited at the herbarium of the Instituto Brasileiro de Geografia e Estatística.

Extraction and isolation. The air-dried powdered roots (3.0 kg) were extracted exhaustively with EtOH in a Soxhlet apparatus. The EtOH solution was concentrated *in vacuo* to yield a residue (320 g). A portion of this residue (21.4 g) was dissolved in hot MeOH and precipitated after addition of EtOAc. The precipitate was filtered and washed with hot EtOAc to yield 5.0 g of material which was chromatographed on a silica gel (200 g) column using CHCl3-MeOH (7:3) as eluent. Twenty two fractions of 50 ml each were collected. Fractions 8 to 11 afforded **1** (2.5 g) as an amorphous solid colourless, after drying and trituration.

3-O-[α-L-Rhamnopyranosyl (1→2)-b-D-xylopyranosyl (1→2)β-D-xylopyranosyl]-16β,23(R):16α,30 α-diepoxy-20(S)-hydroxy-dammar-24-ene (**1**). Mp 188-200 °C, colourless amorphous solid. IR ν^{KBr}_{max} cm^{-1}: 3400 (OH), 1680, 840 (CH=C), 1150, 1080, 1040 (C-O). FABMS [($SCH_2CH_2OH)_2$ matrix, negative ion mode] *m/z* (rel. int.) 882 (100, M⁺), 736 (15, **7**), 266 (22, **7a**), 185 (41, **8**).

Acetylation of compound **1**. The peracetyl derivative **1a** was prepared by treatment of [1] (110 mg) with Ac2O (2.5 ml) and pyridine (1 ml). The usual work-up, after the solution had been allowed to stand for 12 hr at room temperature, followed by chromatography on a silica gel column gave peracetyl derivative **1a** (106 mg), mp 142-144 °C. IR ν^{KBr}_{max} cm^{-1}: 1750 (C=O ester), 1240, 1220, 1090, 1050 (C-O). ^{1}H NMR: Table I. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): 489 (6, **2**), 437 (6, **3**), 274 (13), 273 (99, **4**), 259 (13), 213 (24, **5**), 201 (7), 199 (6), 187 (7), 173 (5), 171 (27), l69 (6), 161 (14), 159 (6), 157 (27), 154 (9), 153 (100, **6**), 149 (5), 147 (7), 145 (8), 143 (8), 142 (5), 140 (6), 139 (21), 137 (10), 135 (8), 133 (7), 129 (8), 127 (9), 125 (18), 123 (6), 121 (7), 119 (8), 115 (14), 113 (7), 112 (6), 111 (62), 109 (16), 107 (10); FABMS [($SCH_2CH_2OH)_2$ matrix, negative ion mode] *m/z* (rel. int.): 1328 [34, M+ ($SCH_2CH_2OH)_2$-2H], 1208 [11, M+ (SCH_2CH_2OH-2H-2AcOH], 1153[19, M+ (SCH_2CH_2OH)-2H-2AcOH-CH=CMe_2], 405 (2,8-tri-O-acetyl-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl-OH), 266 (53, **7a**), 185 (100, **8**).

Acid hydrolysis of **1**. Compound **1** (250 mg) was dissolved in 20 ml of MeOH-H_2O (1:1) with concentrated HCl (10 ml) and the solution was refluxed for 2 hr. The mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anydrous Na_2SO_4 and concentrated under reduced pressure to yield an impure aglycone. The H_2O solution was concentrated, under reduced pressure, to dryness after addition of acetone and the sugar were identified as rhamnose and xylose (1:2) by comparison with authentic samples by thin layer chromatography (TLC) using silica gel G (Merck) impregnated with 5% of NaOAc[11] as adsorbent and EtOAc-*iso*PrOH-H2O (3.5:3.9:2.6) as eluent. Spots were visualized by spraying with a freshly pre pared solution of diphenylamine (4% in EtOH), aniline (4% in EtOH), and concentrated phosphoric acid (5:4:1), after heating for 10 min[12].

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Table 1. ^{1}H and ^{13}C chemical shifts values (δ)[a] of the sugar moiety of peracetyl **1a** and **1** compared with the model **13**[3].

	1		**13**[c]				
	δ_H[b]		δ_C	δ_C	δ_H[b]	δ_C	δ_H
C	$CDCl_3$	C_6D_6	$CDCl_3$	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N
1'	4.46(d,J=5.9)	4.70(d,J=5.7)	103.47	106.62	4.86(d,J=6.7)		
2'	3.80(dd,J=5.9,8.4)	4.2-3.9	74.51	80.93	4.2		
3'	5.2-5.0	5.43(t,J=8)	69.14	79.29	4.2		
4'	5.0-4.8	5.2-5.0	72.76	72.72	4.6		
5'	3.37(t,J=8.6)	3.18(dd,J=8.6,11.6)	62.01	66.91	4.3		
1"	4.2-3.9	4.2-3.9	-	-			
2"	4.63(d,J=6.6) 5.70(d,J=7.6)	4.79(d,J=6.2)	100.94	103.19	5.72(d,J=6.6)	102.60	
3"	3.57(dd,J=6.6,8.6)	3.82(dd,J=6.2,8.3)	75.85	80.93	4.3	79.50	
4"	5.2-4.9	5.41(t,J=8.3)	69.36	78.52	4.7	78.24	
5"	5.0-4.8	5.2-5.0	72.76	71.68	4.1	70.90	
1"'	3.31(t,J=8.6)	3.31(dd,J=7.2,11.3)	61.48	66.91	4.3	66.90	
2"'	4.2-3.9	4.2-3.9	-	-			
3"'	4.89(br s)	5.24(br s)	97.94	102.31	6.35(s)	102.40	6.37(br s)
4"'	5.07(br s)	5.50(d,J=2.9)	69.78	72.52	4.7	72.40	
5"'	5.2-4.9	5.67(dd,J=2.9,9.6)	67.87	72.72	4.6	72.40	
6"'	5.2-4.9	5.49(t,J=9.6)	70.65	74.43	4.3	74.36	
OAc	4.1-3.9	4.47(dq,J=9.6,6.1)	66.84	69.72	4.9	69.51	
	1.15(d,J=5.8)	1.53(d,J=6.1)	17.09	19.09	1.79(d,J=5.9)	18.91	
	2.08(s)	2.01(s)	170.24	-	-		
	2.06(s)	1.97(s)	170.11	-	-		
	2.05(s)	1.79(s)	169.88	-	-		
	2.01(s)	1.77(s)	169.60	-	-		
	1.99(s)	1.77(s)	20.72	-	-		
	1.97(s)	1.72(s)	20.67	-	-		
	1.94(s)	1.72(s)	20.60	-	-		

a Chemical shifts in d (ppm) and TMS as internal standard. Coupling constants (J) in Hz. The assignments were made with the aid of the DEPT and 2D-shift-correlated (1H x 1H-COSY and 1H x 13C-COSY) spectra (except for acetyl groups).

b 1H chemical shifts described with only one decimal correspond to approximate values of **1** and **1a** obtained of the 1H x 13C-COSY spectra.

c We described only useful values for comparison.

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NOTAS

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Table 2. ^{1}H and ^{13}C NMR chemical shifts values (d)[a] of the aglycone of peracetyl **1a** and **1** compared with the models **9**[4], **10**, **12**[7] and **11**[5].

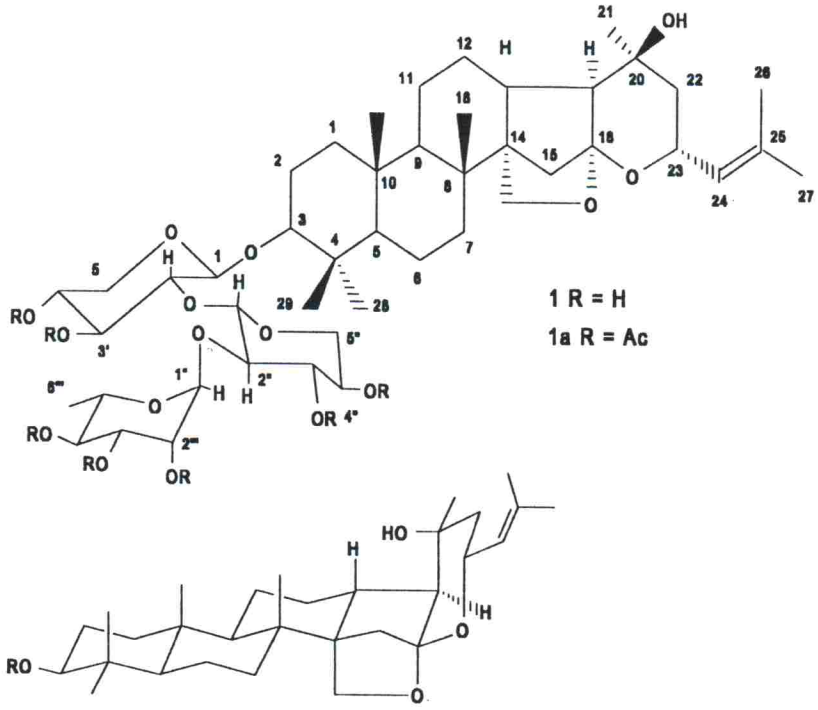
	1a($CDCl_3$)		**1**(C_5D_5N)		**9**[b]	$CDCl_3$	C_5D_5N	
C	δ^C	δ^H	δ^C	δ^H	δ^C	**10**	**11**[c]	**12**[c]
1	38.49		39.90			39.00	39.0	39.5
	33.7							
2	25.85		27.03			26.70	27.4	26.8
	25.5							
3	89.69	3.1-2.9	89.54	3.25(m)		88.10	78.9	89.5
	75.9							
4	39.37	-	40.11	-		39.70	39.0	39.8
	37.6							
5	55.89	0.7	56.48	0.7		56.30	55.9	56.7
	49.5							
6	17.88		18.64			18.30	18.3	18.6
7	35.52		37.33			36.00	35.3	35.3
8	37.13	-	37.49			37.30	40.4	40.3
9	52.51	0.8	53.29	0.8		53.00	50.7	50.4
10	36.98	-	37.49	-		37.30	37.1	37.2
11	21.30		22.03			21.80	21.6	
12	27.91		28.77			28.60	25.4	
13	36.85		38.96	2.80(m)		35.70	42.3	
14	53.29	-	53.96			53.70	50.3	
15	35.39	1.6-1.4	37.77	2.48(d,J=11.7)		37.10	31.2	
16	109.48	-	110.87	-		110.60	27.6	
17	52.62	1.1	54.15	1.4		53.90	49.9	
18	18.51	1.06(s)	19.15	1.04(s)		18.30	16.2*	16.2*
19	15.88	0.77(s)	16.58	0.77(s)		16.40	15.5*	16.2*
20	69.23	-	68.72	-		68.50	75.4	
21	29.84	1.14(s)	30.27	1.29(s)		30.00	24.9	
22	44.36	1.6-1.4	45.60	1.7		45.30	40.5	
23	68.60	4.60	68.93	5.27(dd,J=8.9,6.0)		68.50	22.6	
24	125.04	5.15	127.21	5.51(d,J=6.0)		127.00	124.8	
25	135.28	-	134.56	-		134.2	131.5	
26	18.26	1.63(br s)	19.09	1.66(s)		25.50	17.7	
27	25.51	1.67(br s)	25.91	1.64(s)		18.90	25.7	
28	27.60	0.94(s)	28.77	1.22(s)		28.00	28.0	28.2
	28.5							
29	16.02	0.80(s)	16.71	1.04(s)		16.80	15.4*	16.6*
	22.1							
30	65.72		66.12			65.8	16.5	

a Chemical shifts in d (ppm), coupling constants (J) in Hz and TMS as internal standard. Assignments were made with the aid of the DEPT and 2D-shift-correlated [1H x 13C-COSY, optimized for one-bond couplings (1JCH)] spectra. 1H Chemical shifts described with only one decimal correspond to approximate values of Ia and **1** and may correspond to only one proton in the case of the methylene groups.

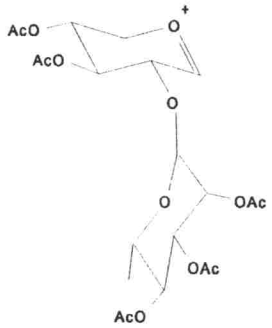
b The assignments of carbon atoms C-7, C-8, C-10, C-13, and C-15 need to be re-examined.

c We described only useful values for comparison.

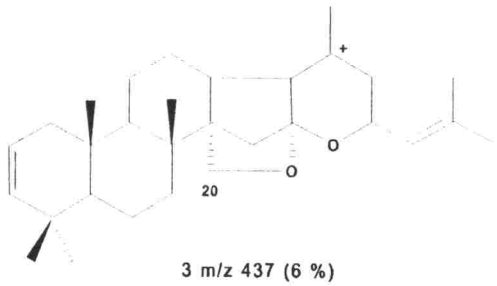
* From the authors of the paper: "values in any vertical column may be reversed although those given here are preference".



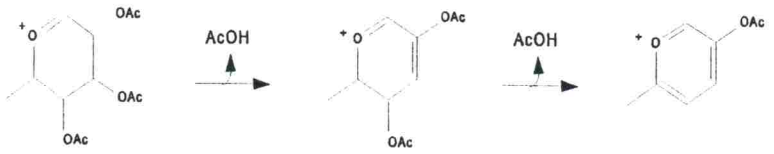
1b



2 m/z 489 (6 %)



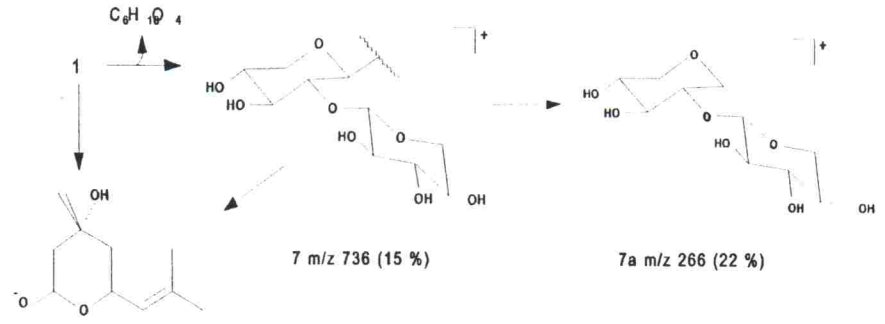
3 m/z 437 (6 %)



4 m/z 273 (99 %)

5 m/z 213 (24 %)

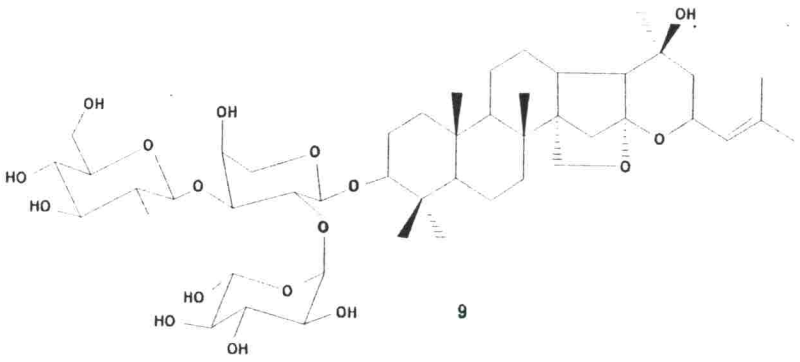
6 m/z 153 (100 %)



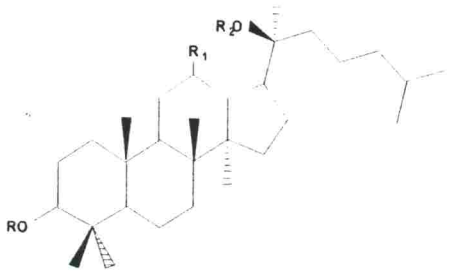
8 m/z 185 (41 %)

7 m/z 736 (15 %)

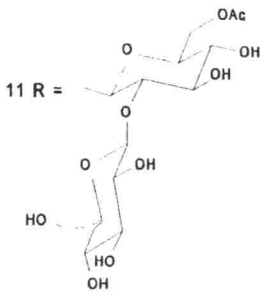
7a m/z 266 (22 %)



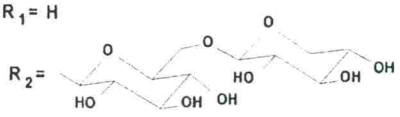
9



10 R = R_1 = R_2 = H



11 R =



R_1 = H

R_2 =

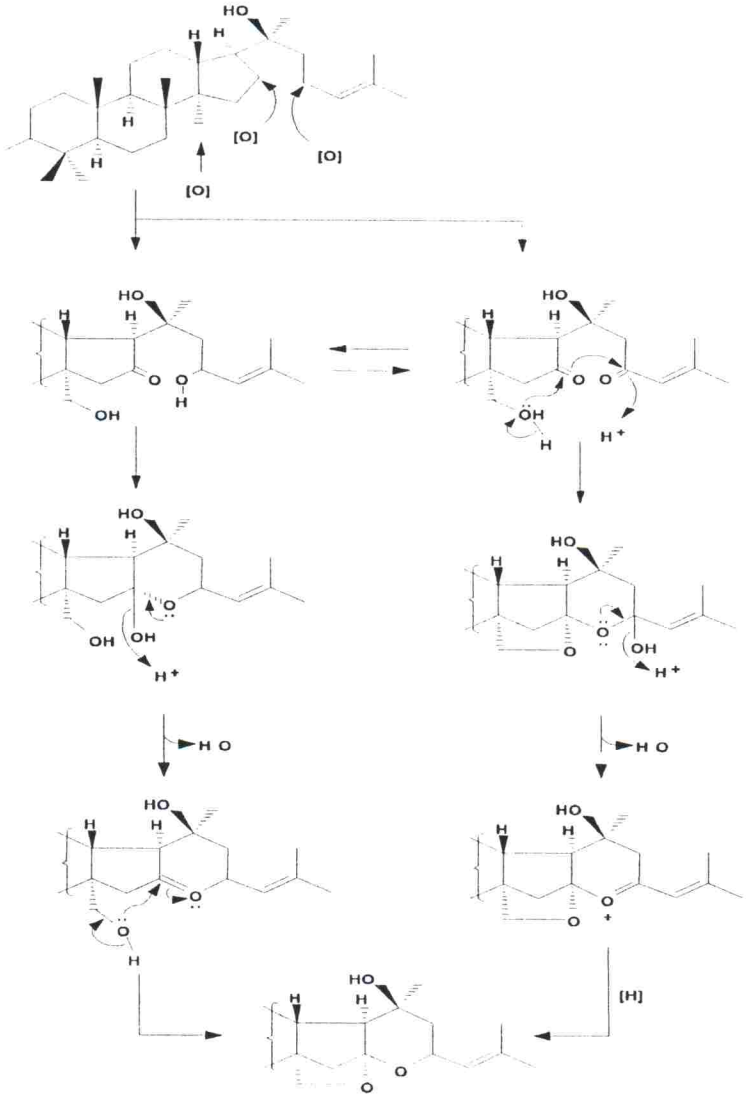


Chart 1. Suggested biosynthetic pathway for the formation of the two heterocyclic rings in 1.