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SPECTROSCOPIC STUDIES ON A WITHANOLIDE FROM *WITHANIA COAGULANS*

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Key Word Index—*Withania coagulans*, Solanaceae, withanolides, steroidal lactones, trichloroacetylcarbamate esters, sitosterol glucoside

Abstract—The structure of a new withanolide was elucidated as 3β,14α,20α_F,27-tetrahydroxy-1-oxo-20*R*,22*R*-witha-5,24-dienolide using chemical and spectroscopic methods The structure was corroborated by comparative studies with known closely related withanolides Sitosterol-β-D-glucoside was identified through chemical and spectroscopic means

INTRODUCTION

A chemical examination of the fruits of *Withania coagulans* has been reported in a previous paper which describes some new biogenetic precursors of withanolides [1] Further studies of the extract furnished a new withanolide **1a** along with sitosterol-β-D-glucoside (stigmast-5-en-3β-ol-β-D-glucoside) in minor quantities The structure of compound **1a** has been elucidated as 3β,14α,20α_F,27-tetrahydroxy-1-oxo-20*R*,22*R*-witha-5,24-dienolide† or 3β-hydroxy-2,3-dihydrowithanolide H

RESULTS AND DISCUSSION

Compound **1a** analysed for $C_{28}H_{40}O_7$ and showed in the UV spectrum an absorption at λ_{max} 218 nm (ε 9800) The sizeable blue shift compared to the characteristic absorption (225 nm) of the usual dimethyl substituted α,β-unsaturated δ-lactone of the withanolides, indicates its presence with an α-hydroxymethyl substitution [2] The IR spectrum showed the presence of several hydroxyl groups (3580 and 3410 cm^{-1}), a six-membered ring ketone (1705 cm^{-1}), an α,β-unsaturated δ-lactone (1690 cm^{-1}) and double bond (1650 cm^{-1})

The ^1H NMR spectrum (Table 1) had the general features of withanolides Four singlets at δ1 06, 1 30, 1 33 and 2 05 accounted for four methyl groups The upfield signal at δ1 06 was assigned to the 18-Me with a 14α-hydroxyl substitution and a 17β-oriented side chain by comparison with similarly substituted withanolides, like withanolide H (**2a**) and related compounds [3–6] The low field singlet at δ2 05 was assigned to the 28-Me being attached to the unsaturated δ-lactone The absence of a second signal corresponding to the 27-Me and the presence of a broad two protons signal at δ4 37 implied that this position is substituted with a hydroxymethyl group The signals at δ1 30 and 1 33 were thereby assigned respectively to the 21-Me (adjacent to a hydroxyl) and the 19-Me (vicinal to the 1-keto group) in conformity with similarly substituted withanolides The characteristic double doublet at δ4 28 was related to the 22-H of the 17β-oriented side chain In the lowfield, the one proton broad doublet at δ5 66 was assigned to the 6-H of the Δ^5-bond A broad multiplet at δ3 87 was for the proton adjacent to the secondary hydroxyl group No signals could be seen for the characteristic Δ^2-en-1-one system of the withanolides, and therefore a 1-keto-3-hydroxyl substitution was indicated for ring A This system was also supported by observing a clean double doublet at δ2 74 and 2 66 for the axial 2β-H and the equatorial 2α-H respectively

Further evidence for the presence of the primary 27-hydroxyl and the secondary 3-hydroxyl was adduced by the analysis of the ^1H NMR spectrum of the acetate **1b** It displayed two additional singlets at δ2 05 and 2 07 for the two newly formed acetoxyl groups Here also a double doublet at δ4 27 for 22-H, and a broad singlet at δ5 70 for 6-H were present The two 27-methylene protons adjacent to the acetoxy group shifted down field by 0 52 ppm, and an expected similar shift of 1 00 ppm for the 3α-H took place, when compared to the original compound The latter downfield shift and its multiplicity confirmed the presence and equatorial orientation of the 3β-hydroxyl Two neat double doublets at δ2 75 (J = 13 4, 6 2 Hz) and δ2 67 (J = 13 4, 5 4 Hz) were assigned to the 2β-H_{ax} and 2α-H_{eq} respectively Also a double doublet at δ2 65 (J = 13 4 and 8 4 Hz) was well seen for the 4β-H_{ax} Indeed, upon irradiation at the location of the 3α-H signal (δ4 88) all three double doublets collapsed into three clear doublets, confirming their respective assignments The UV absorption was λ_{max} 216 nm for the 27-acetoxylated product, showing a slight hypsochromic shift [2] The IR spectrum, showed two additional bands at 1730 and 1710 cm^{-1} for the diacetate **1b**

Pyridine is known to form weak hydrogen bonding and collision complexes with hydroxyl groups, and thereby produces shielding and deshielding cones and influences the chemical shift of the neighbouring protons [7] The shift differences observed are then useful in locating positions of hydroxyls and neighbouring groups [8] The

†Semi-systematic nomenclature used 'withanolide' refers to 22-hydroxyergostan-26-oic acid δ-lactone

Table 1 ^{1}H NMR signals of relevant protons in compounds **1** and **2**

Compound	2-H	3-H	6-H	15-H	22-H	Methyl groups 18	19	21	28	Other signals
	2 74 H-β (*dd*, 14, 5 9) 2.66 H-α (*dd*, 14, 2 6)	3 87 (*m*, *W* 20)	5 66 (*br d*, *W*9)	—	4 28 (*dd*, 13, 4)	1 06 (*s*)	1 33 (*s*)	1 30 (*s*)	2 05 (*s*)	4 37 (*br dd*, *W* 12 2, 27-C<u>H</u>$_2$OH
1a	[3 03] H-β (*dd*, 12 5, 8 5) [2 94] H-α (*dd*, 12 5, 5 7)	[4 17] (*m*, *W*17)	[5 70] (*br s*, *W*9)	—	[4 48] (*dd*, 13 1, 2 8)	[1 36] (*s*)	[1 41] (*s*)	[1 49] (*s*)	[1 89] (*s*)	[4 83] (*d*, 12 0, 27-H$_a$C<u>H</u>$_b$OH) [4 70] (*d*, 12 0, 27-<u>H</u>$_a$CH$_b$OH)
	2 75 H-β (*dd*, 13 4, 6 2) 2 67 H-α (*dd*, 13 4, 5 4)	4 88 (*m*, *W*16)	5 70 (*br s*, *W*9)	—	4 27 (*dd*, 12 9, 3 8)	1 06 (*s*)	1 30 (*s*)	1 31 (*s*)	2 09 (*s*)	4 89 (*br d*, *W*7 1, 27-C<u>H</u>$_2$OAc) 2 05 (*s*, 3β-O<u>Ac</u>) 2 07 (*s*, 27-O<u>Ac</u>) 2 65 (*dd*, 13 4, 8 4, 4β-H)
1b	[2 87] H-β (*dd*, 13, 6 5) [2 83] H-α (*dd*, 13, 4)	[5 15] (*m*, *W*18)	[5 66] (*br s*, *W*8)	—	[4 53] (*dd*, 13 1, 3 5)	[1 37] (*s*)	[1 37] (*s*)	[1 50] (*s*)	[1 82]	[5 19] (*d*, 12 0, 27-<u>H</u>$_a$CH$_b$OAC) [5 09] (*d*, 12 0, 27-H$_a$ C<u>H</u>$_b$OAc) [2 01] (*s*, 27-O<u>Ac</u>) [1 98] (*s*, 3β-O<u>Ac</u>)
1c	—	4 95 (*m*, *W*21)	5 77 (*br dd*, *W*9)	—	4 43 (*dd*, 14, 6 7)	1 16 (*s*)	1 31 (*s*)	1 86 (*s*)	2 21 (*s*)	5 09 (*br s*, *W*4, 27-C<u>H</u>$_2$OTAC) 8 58 (*s*, 27-O<u>TAC</u>) 8 37 (*s*, 3β-O<u>TAC</u>) 8 30 (*s*, 14-O<u>TAC</u> and 20-O<u>TAC</u>)
1d	—	4 95 (*m*, *W*21)	5 77 (*br dd*, *W*9)	5 29 (*br dd*, *W*7)	4 43 (*dd*, 14, 6 7)	1 12 (*s*)	1 31 (*s*)	1 86 (*s*)	2 21 (*s*)	5 09 (*br s*, *W*4, 27-C<u>H</u>$_2$OTAC) 8 58 (*s*, 27-O<u>TAC</u>) 8 37 (*s*, 3β-O<u>TAC</u>) 8 30 (*s*, 20-O<u>TAC</u>)

1e	—	4 88 (*br m*, *W*22)	5 72 (*br d*, *W*10)	—	4 44 (*dd*, 14 4, 6 7)	1 16 (*s*)	1 31 (*s*)	1 86 (*s*)	2 11 (*s*)	2 05 (*s*, 3β-OAc) 2 07 (*s*, 27-OAc) 4 89 (*br d*, *W*6 4, 27-C$\underline{H}_2$OAc) 8 32 (*s*, 14-O$\underline{TAC}$ and 20-O$\underline{TAC}$)
1f	—	4 88 (*br m*, *W*22)	5 72 (*br d*, *W*10)	5 27 (*br s*, *W*6)	4 44 (*dd*, 14 4, 6 7)	1 12 (*s*)	1 31 (*s*)	1 86 (*s*)	2 11 (*s*)	2 05 (*s*, 3β-OAc) 2 07 (*s*, 27-OAc) 4 89 (*br d*, *W*6 4, 27-C$\underline{H}_2$OAc) 8 32 (*s*, 20-O$\underline{TAC}$)
2a	5 89 (*d*, 9 8)	6 79 (*ddd*, 9, 4 5, 2 3)	5 62 (*br d*, *W*12)	—	4 29 (*dd*, 13, 3 5)	1 08 (*s*)	1 27 (*s*)	1 32 (*s*)	2 06 (*s*)	4 39 (*br s*, *W*8 5, 27-C$\underline{H}_2$OH)
2b	5 89 (*d*, 9 8)	6 80 (*ddd*, 9, 4 5, 2 3)	5 61 (*br d*, *W*11)	—	4 43 (*dd*, 14 1, 7)	1 17 (*s*)	1 25 (*s*)	1 85 (*s*)	2 20 (*s*)	5 09 (*br s*, *W*3 2, 27-C$\underline{H}_2$OTAC) 8 30 (*s*, 14-O$\underline{TAC}$ and 20-O$\underline{TAC}$) 8 56 (*s*, 27-O$\underline{TAC}$)
2c	5 89 (*d*, 9 8)	6 80 (*ddd*, 9, 4 5, 2 3)	5 61 (*br d*, *W*11)	5 26 (*br s*, *W*6)	4 43 (*dd*, 14 1, 7)	1 12 (*s*)	1 25 (*s*)	1 85 (*s*)	2 20 (*s*)	5 09 (*br s*, *W*3 2, 27-C$\underline{H}_2$OTAC) 8 30 (*s*, 20-O$\underline{TAC}$) 8 56 (*s*, 27-O$\underline{TAC}$)

Chemical shifts are in δ units measured in $CDCl_3$, and the data in square brackets are for pyridine-d_5 solutions, coupling constants (in Hz) are in parentheses, *s*-singlet, *d* = doublet, *dd* = double doublet, *ddd* = double double doublet, *br* = broad, *m* = multiplet, *W* refers to $W_{1/2}$, $\underline{TAC}$ for –CONH$\underline{C}$OCCl$_3$

differences ($\Delta = \delta CDCl_3 - \delta C_5D_5N$) in compound **1a**, for 18-Me (−0 3 ppm), 21-Me (−0 19) and 22-H (−0 22) were measured due to the influence of the 20α_F-hydroxyl group Remarkably, in pyridine-d_5 the 27-methylene protons separated into a neat pair of doublets and appeared at δ 4 83 and 4 70, recording a difference of −0 46 and −0 33 ppm respectively The 3β-hydroxyl induced shift differences in 3α-H_{ax} of −0 30, 2β-H_{ax} of −0 29 and 2α-H_{eq} of −0 28 Interestingly, the 27-hydroxyl induced in this case an upfield shift of +0 16 for the 28-Me The 19-Me was not much affected (Δ = −0 08 ppm) since it is located fairly far from the 3β-hydroxyl A similar type of shift differences was also observed in **1b**, as presented in Table 1

Trichloroacetyl isocyanate (TAI) is presently a well known reagent reacting with hydroxyl groups to form a carbamate ester (–O CO NH CO CCl_3, OTAC) in which the imide protons could easily be located at lowfield δ8–9 in the ^{1}H NMR spectrum [4, 8, 9] Compound **1a** was found to be an interesting case containing primary, secondary and tertiary alcohol functions Therefore the use of TAI could provide interesting observations and contribute in determining unequivocally the respective positions of the hydroxyl groups It has been previously reported that the 14α-carbamate seems to be very labile and readily eliminates to form a 14/15 double bond [4] On these grounds a ^{1}H NMR monitored time experiment was carried out on compound **1a** by adding a few drops of TAI to a solution in the NMR tube, and rapidly measuring spectra at predetermined time intervals The rapid formation of the lowfield imide protons was observed together with the appearence of the vinylic 15-H and the respective shifts of the Me-signals involved in the reaction During the first 3 min, TAI reacted completely with the 3β-,14α- and 27-hydroxyls and showed three imide protons (**1c**) The ester formation obviously influenced the methyl signal positions A downfield shift of 0 10 ppm was observed for 18-Me and of 0 16 ppm for 28-Me The 20-OTAC (**1c**) was found to form only to an extent of 30% since two methyl signals were seen at δ 1 34 and 1 86 in a relationship of 7 to 3 for the 21-Me, reflecting the 20-hydroxyl and 20-OTAC respectively Even during the first 3 min, 35% of 14-OTAC had eliminated to form the Δ^{14}-bond, showing a small signal at δ 5 26 for the 15-H The presence of the partial 18-Me signal at δ 1 12 (upfield by 0 04 ppm) compared to that with 14-OTAC was a good sign of the influence of the 14/15 double bond on the location of the 18-Me signal After 5 min, 14-OTAC had completely eliminated, as indicated by the total disappearance of the δ 1 16 signal, whereas the new signal at δ 1 12 had reached its maximum size, as had the 15-H vinylic proton for the Δ^{14}-bond During this time, the 20-OTAC had already formed to an extent of 70%, as indicated by the respective intensities of the δ1 86 and 1 34 signals for the 21-Me In about 30 min 20-OTAC (**1d**) was completely formed and only one full signal at δ1 86 was present After two days, a similar spectrum was obtained to that for the 30 min product

In order to detect the influence of the carbamate ester on the 28-Me, a similar TAI reaction was carried out on the diacetate **1b** However, the 28-Me did not shift downfield as had happened in compound **1a** Similar observations were noticed concerning 14α- and 20-hydroxyls, producing **1e** and finally **1f**, and their ^{1}H NMR data are given in Table 1

To corroborate these observations, withanolide H (**2a**), obtained from previous work [5], having close structural resemblances with **1a** was studied under similar conditions with TAI and ^{1}H NMR monitoring The same behaviour was observed, whereby a tricarbamate **2b** was formed first within 3 min followed by gradual elimination at C-14 to produce finally the Δ^{14}-derivative **2c** identified by a full one proton signal at δ 5 26 for 15-H (Table 1) The ^{1}H NMR data for withanolide H (**2a**) are now being given since they were previously reported only for its acetate [3, 4]

For the ^{13}C NMR signal assignments of **1a** and **1b** (Table 2), the data were compared with those previously

Table 2 ^{13}C NMR spectral data of compounds **1a**, **1b** and **2a**

Carbon	1a*	1b	2a†	Carbon	1a*	1b	2a†
1	212 2	210 3	204 2	17	49 4	49 5	49 4
2	52 9	44 0	128 0	18	17 4	17 3	17 5
3	68 7	70 0	145 5	19	19 1	18 4	19 0
4	41 0	37 2	33 5	20	75 2	75 4	75 3
5	134 9	134 3	135 2	21	21 1	21 0	21 1
6	126 0	126 5	125 0	22	82 0	81 3	81 7
7	25 6	25 6	25 3	23	31 9	32 3	31 9
8	35 9	36 3	35 2	24	154 7	156 9	153 7
9	34 1	34 0	36 3	25	125 5	122 0	125 6
10	51 3	53 0	50 9	26	166 9	164 5	166 4
11	20 9	20 9	22 2	27	56 6	58 0	57 1
12	32 1	29 7	32 5	28	20 1	20 7	20 1
13	47 5	47 7	47 4	Me CO at	—	170 9	—
14	84 9	84 9	85 1	Me CO C-27	—	21 2	—
15	31 9	32 3	31 9	Me CO at	—	170 2	—
16	20 8	20 8	20 7	Me CO C-3	—	21 2	—

*A few drops of MeOH were added to improve solubility
†Data from ref [10]

given for the carbons of withanolide H (**2a**) and its Δ^3-isomer [10] For easier comparison, the values reported for **2a** have been included in Table 2 It can be seen that except for the signals of carbons C-1 (sp^2), C-2, C-3 and C-4 respectively δ 212 2, 52 9, 68 7 and 41 0, all other values are alike In the acetate **1b**, the 3-acetoxyl induced a slight upfield shift of the C-1 carbonyl carbon, probably due to the absence of interaction between the 3-hydroxyl and the C-1 oxygen, and the lack of the more polar 3β-hydroxyl Similar shielding effects were also observed for C-2 and C-4 The 27-acetoxyl has increased the polarity of the 24/25-double bond and thereby caused a downfield shift by 2 2 ppm for the 24- and an upfield shift by 3 5 ppm for the 25-sp^2 carbons The assignments for the A/B ring carbons for compound **1a** and its acetate **1b** are further supported by the similarities found with the data provided for 3β,14α,17β,20α_F-tetrahydroxy-1-oxo-20*S*,22*R*-witha-5,24-dienolide and its acetate, reported in the preceding work on this plant [1, compounds **1a** and **1b** in that paper]

The mass spectra of compound **1a** and its acetate **1b** under electron impact have not shown the molecular ion peaks Nevertheless, the fragmentation pattern described for compound **1a** (Scheme 1) and compound **1b** (Experimental section) is in agreement with the general features of mass fragmentation of withanolides For instance, the side chain cleavage at C-17 to C-20 and C-20 to C-22 producing different fragments, as well as a new cleavage of ring A and its further fragmentation by subsequent loss of water molecules and methyl groups were observed

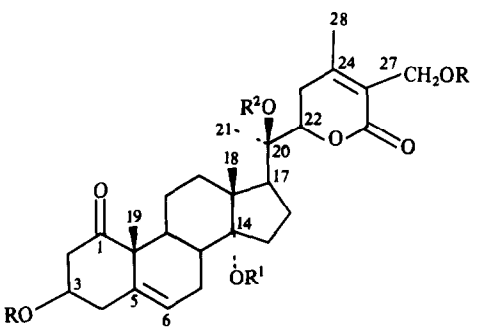
The presence of a 1-keto-3-hydroxyl system in compound **1a**, is the second case for the occurrence of such a system in our studies on *W coagulans* To the best of our knowledge, only once before has such a system been referred to in a plant [6] Presently, a precursor for the 2-en-1-one system of the withanolides is being fully described both for compounds having the side chain α-oriented [1] and here for a regular β-oriented side chain Once again the presence of 1-keto-3-hydroxyl system, follows the biogenetic sequence described in the preceding paper [1] Furthermore, it is our present belief that the elimination of 3-hydroxyl (as H_2O) can take place along two lines one by elimination towards C-2 to produce the 2-en-1-one system, forming withanolide H (**2a**) or secondly, towards C-4 to produce the 3-en-1-one system (conjugated to Δ^5) producing the Δ^3-isomer of withanolide H (compound **2** in ref [6]) The presence of the latter system was also found in other withanolides, isolated from *W somnifera* [3, 4]

The other minor compound analysed for $C_{35}H_{60}O_6$ and gave a positive Molisch test for glycosides Upon acetylation it formed a tetraacetate The ^{1}H NMR spectrum of the acetate accounted for all the signals for sitosterol-β-D-glucoside tetraacetate (see Experimental) The chemical shift values of the aglycone methyl groups are in conformity with the reported values for sitosterol [11] The signal assignments for the sugar protons are supported by appropriate spin decoupling experiments and correct integration The mass spectrum of the glycoside did not show the molecular peak, but the glycoside link cleavage and aglycone and sugar fragmentations were observed Finally, the acid hydrolysis of the glycoside furnished sitosterol and D-glucose, which were identified through authentic samples via mmp and co-TLC Mp and $[\alpha]_D$ values of the glycoside are identical with those reported for sitosterol-β-D-glycopyranoside [12]

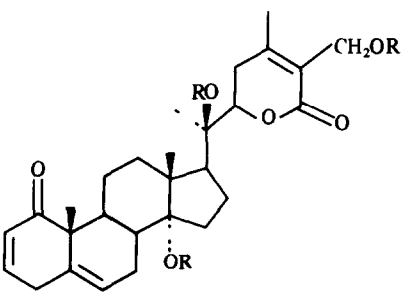
EXPERIMENTAL

Mps were measured on a Fischer-Johns apparatus and are uncorr UV spectra were measured for EtOH solns ^{1}H NMR and ^{13}C NMR spectra were determined with Bruker WH270 and WH90 (operating at 22 63 MHz) instruments respectively for $CDCl_3$ solns with TMS as internal standard Analytical TLC was carried out using chromatoplates (50 × 75 mm, silica gel F_{254}) MS were determined under the direction of Dr Z Zaretskii, and microanalyses were carried out by Mr R Heller of the Weizmann Institute of Science

Isolation procedure The procedure described earlier for *Withania coagulans* Dunal [1], using 5 kg of dried powdered fruits, was further continued to examine the unidentified minor compounds present in the EtOAc and EtOAc–MeOH fractions obtained by CC The former fractions on evaporation to dryness under vacuum gave a gummy residue, which was purified by passing through a second short column of silica gel H, using EtOAc as eluent The fractions containing the same compound

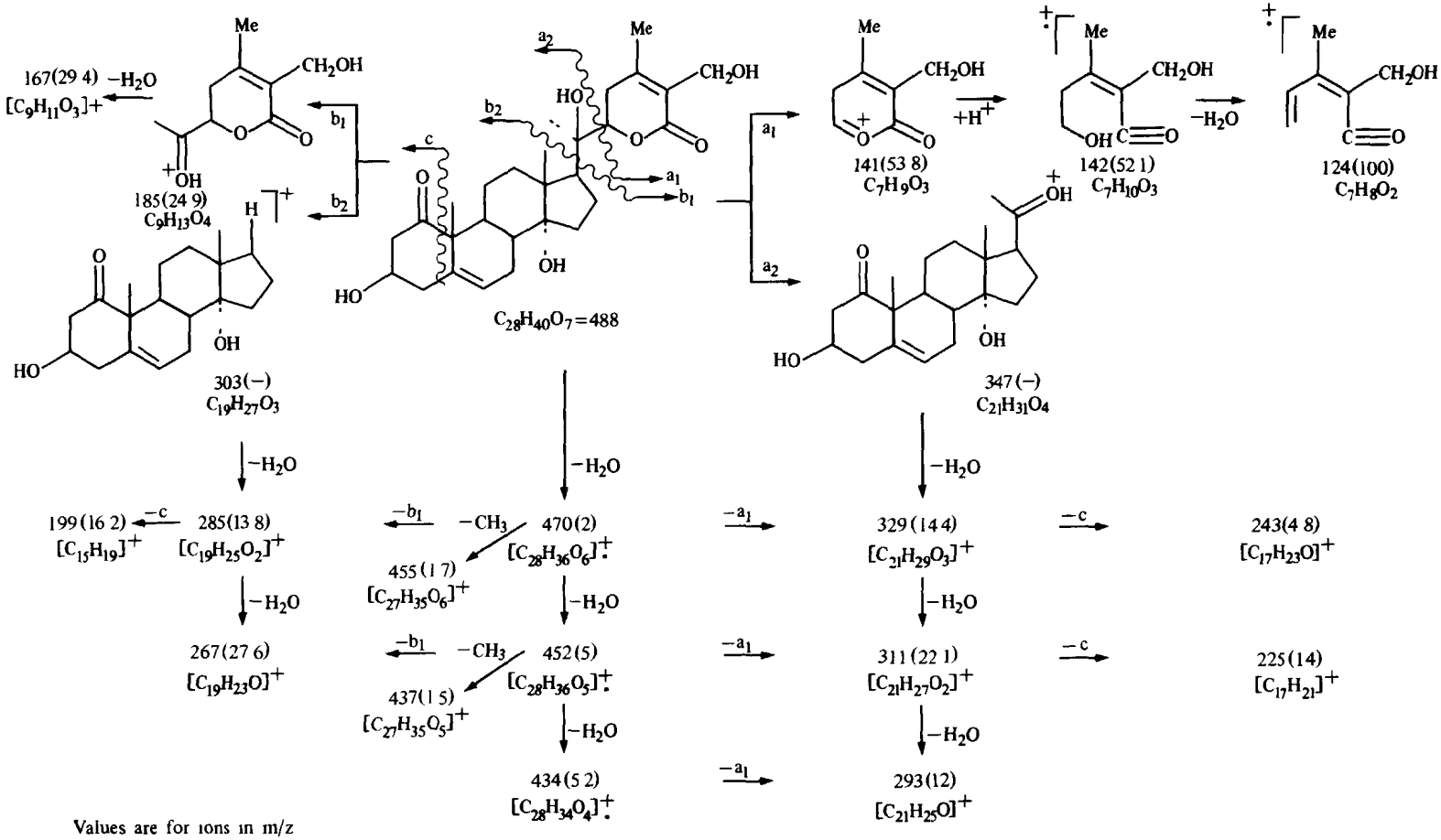


1a R = R^1 = R^2 = H
1b R = Ac, R^1 = R^2 = H
1c R = R^1 = R^2 = TAC
1d Δ^{14}, R = R^2 = TAC
1e R = Ac, R^1 = R^2 = TAC
1f Δ^{14}, R = Ac, R^2 = TAC



2a R = H
2b R = TAC
2c Δ^{14}, R = TAC

TAC = -CO NH CO CCl_3


167(29 4) −H2O
[C9H11O3]+
185(24 9)
C9H13O4
303(−)
C19H27O3
C28H40O7=488
141(53 8)
C7H9O3
142(52 1)
C7H10O3
124(100)
C7H8O2
347(−)
C21H31O4
199(16 2)
[C15H19]+
285(13 8)
[C19H25O2]+
267(27 6)
[C19H23O]+
455(1 7)
[C27H35O6]+
470(2)
[C28H36O6]+
437(1 5)
[C27H35O5]+
452(5)
[C28H36O5]+
434(5 2)
[C28H34O4]+
329(14 4)
[C21H29O3]+
311(22 1)
[C21H27O2]+
293(12)
[C21H25O]+
243(4 8)
[C17H23O]+
225(14)
[C17H21]+
Values are for ions in m/z
In parentheses abundance of fragment %

Scheme 1

(monitored by TLC) were combined and upon evaporation gave a solid substance, which was purified by several crystallizations from Me_2CO–EtOAc, yielding compound **1a** (60 mg) The EtOAc–MeOH fractions were evaporated under vacuum and the residue following several crystallizations from absolute EtOH yielded sitosterol-β-D-glucoside (65 mg)

3β,14α,20α$_F$,27-Tetrahydroxy-1-oxo-20R,22R-witha-5,24-dienolide (**1a**) Mp 190–192° (crystallized from EtOAc–MeOH), $[\alpha]_D$ +64 7° (*c* 0 34, $CHCl_3$–MeOH, 4 1), TLC R_f 0 22 (EtOAc–MeOH, 19 1), UV λ_{max} 218 nm (ε 9760), IR ν_{max}^{KBr} cm^{-1} 3580, 3410, 1705, 1690, 1650 and 800 (Found C, 68 76, H, 8 30 $C_{28}H_{40}O_7$ requires C, 68 83, H, 8 25%, MW 488 6)

3β,27-Diacetoxy-14α,20α$_F$-dihydroxy-20R,22R-1-oxowitha-5,24-dienolide (**1b**) Compound **1a** (40 mg) was acetylated with Ac_2O and pyridine at room temp for 24 hr and after usual work-up compound **1b** was obtained in 90% yield Mp 172–173° (crystallized from $CHCl_3$–EtOAc), $[\alpha]_D$ +50° (*c* 0 83, $CHCl_3$), TLC R_f 0 66 (EtOAc–MeOH 19 1), UV λ_{max} 216 nm (ε 11 070), IR ν_{max}^{KBr} cm^{-1} 3450, 1730, 1710, 1705, 1690, 1650 and 810, MS *m/z* (rel int) 327 (2) $[M-H_2O-227]^+$, 311 (3) $[M-H_2O-AcOH-183]^+$, 293 (3) $[M-2\times H_2O-AcOH-183]^+$, 267 (4) $[M-H_2O-AcOH-227]^+$, 252 (3) $[M-H_2O-AcOH-227-CH_3]^+$, 249 (10), $[M-2\times H_2O-AcOH-227]^+$, 225 (11), $[M-2\times H_2O-183-128]^+$, 224 (2) $[M-H_2O-AcOH-227-CH_3-CO]^+$, 209 (4), [227 (cleavage of C-17 to C-20) $-H_2O]^+$, 199 (2), $[M-H_2O-227-128$ (cleavage of ring A)$]^+$, 183 (6) [cleavage of C-20 to C-22]$^+$ (Found C, 67 09, H, 7 82 $C_{32}H_{44}O_9$ requires, C, 67 11, H, 7 74%, MW 572 7)

Sitosterol-β-D-glucoside Mp 257–258° (becomes brown), 280–283° (dec) (crystallized from absolute EtOH), $[\alpha]_D$ −42 1° (*c* 0 57, pyridine) [12], TLC R_f 0 45 (EtOAc–MeOH, 19 1, spray 5% conc H_2SO_4 in MeOH, pink spot on heating at 110°), IR ν_{max}^{KBr} cm^{-1} 3370, 2930, 2860, 1655, 1640 and 810 UV no strong absorption, MS *m/z* (rel int) 414 (3) $[M-163$ (cleavage of anomeric C–O aglycone, $C_6H_{11}O_5)+H]^+$, 399 (2) $[M-163+H-CH_3]^+$, 398 (5) $[M-163-CH_3]^+$, 397 (19), $[M-179$ (cleavage of glucoside O–C-3 aglycone, $C_6H_{11}O_6)]^+$, 396 (31), $[M-180$ (glucose)$]^+$, 382 (18) $[M-179-CH_3]^+$, 161 (10) $[C_6H_{11}O_6-H_2O]^+$, 145 (13) $[C_6H_{11}O_5-H_2O]^+$, 85 (12) [cleavage of C-22 to C-23]$^+$ (Found C, 72 61, H, 10 53 $C_{35}H_{60}O_6$ requires C, 72 87, H, 10 48%, MW 576 85)

Sitosterol-β-D-glucoside tetraacetate The glucoside was acetylated with Ac_2O–pyridine at room temp for 24 hr and after usual work up gave the tetraacetate Mp 169–170° (crystallized from MeOH), $[\alpha]_D$ −34 5° (*c* 0 39, $CHCl_3$), TLC R_f 0 34 (*n*-hexane–EtOAc, 4 1, spray 5% conc H_2SO_4 in MeOH, brown spot on heating at 110°), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2980, 2900, 1770, 1760, 1750, 1745, 1050 and 920 ^{1}H NMR δ0 67 (3H, *s*, 18-Me), 0 81 (3H, *d*, *J* = 6 8 Hz, 27-Me), 0 83 (3H, *d*, *J* = 6 8 Hz, 26-Me), 0 84 (3H, *t*, *J* = 7 2 Hz, 29-Me), 0 92 (3H, *d*, *J* = 6 5 Hz, 21-Me), 0 99 (3H, *s*, 19-Me), 2 01, 2 03, 2 05, 2 08 (4 × 3H, *s*, O*Ac* at 2',3',4' and 6'), 3 49 (1H, *m*, 3α-H$_{ax}$), 3 67 (1H, *m*, 5'-H), 4 11 (1H, *dd*, *J* = 12, 2 6 Hz, 6'-$\underline{H_a}$–C–$\underline{H_b}$), 4 26 (1H, *dd*, *J* = 12, 4 7 Hz, 6'-H_a–C–$\underline{H_b}$), 4 59 (1H, *d*, *J* = 7 9 Hz, 1'-H anomeric), 4 96 (1H, *dd*, *J* = 9 7, 7 9 Hz, 2'-H), 5 08 (1H, *t*, *J* = 9 5 Hz, 4'-H), 5 21 (1H, *t*, *J* = 9 5 Hz, 3'-H), 5 37 (1H, *br*, $W_{1/2}$ 10 6 Hz, 6-H) (Found C, 69 30, H, 9 24 $C_{43}H_{68}O_{10}$ requires C, 69 32 H, 9 19%, MW 745 00)

Acid hydrolysis of sitosterol-β-D-glucoside The glucoside in EtOH was refluxed with dil HCl (2 N) for 6 hr The reaction mixture was added to H_2O and extracted with Et_2O The EtOH soln was successively washed with aq $NaHCO_3$ and H_2O, and dried over dry $MgSO_4$ Upon evaporation, a white mass separated, which crystallized from MeOH It was identified as sitosterol through mmp 137–138°, and TLC with an authentic sample and its acetate

The aq soln was neutralized with Amberlite IR-45 (OH), filtered and evaporated to dryness *in vacuo* The substance thus obtained was identified as D-glucose with an authentic sample through TLC R_f 0 28 (*n*-BuOH–AcOH–H_2O, 4 1 2 2, spray reagent ammonical $AgNO_3$ and heating to 110°) and co-PC R_f 0 17 (*n*-BuOH–AcOH–H_2O, 4 1 5, spray with aniline hydrogen phthalate prepared from 0 93 g aniline and 1 66 g phthalic acid in 100 ml of *n*-BuOH saturated with H_2O and heating the paper to 100°)

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REFERENCES

1 Vande Velde, V, Lavie, D, Budhiraja, R D, Sudhir, S and Garg, K N (1983) *Phytochemistry* **22**, 2253
2 Lavie, D, Glotter, E and Shvo, Y (1965) *J Org Chem* **30**, 1774
3 Glotter, E, Kirson, I, Abraham, A and Lavie, D (1973) *Tetrahedron* **29**, 1353
4 Kirson, I and Gottlieb, H E (1980) *J Chem Res S*, 388, M, 4275
5 Nittala, S S and Lavie, D (1981) *Phytochemistry* **20**, 2741
6 Vande Velde, V and Lavie, D (1981) *Phytochemistry* **20**, 1359
7 Demarco, D V, Farkas, E, Doddrell, D, Mylari, B L and Wenkert, E (1968) *J Am Chem Soc* **9**, 5480
8 Glotter, E, Kirson, I, Lavie, D and Abraham, A (1971) *J Chem Soc C* 2032
9 Trehan, I R, Monder, C and Bose, A K (1968) *Tetrahedron Letters* 67
10 Gottlieb, H E and Kirson, I (1981) *Org Magn Reson* **16**, 20
11 Rubinstein, I, Goad, L J Clague, A D H and Mulheirn, L J (1976) *Phytochemistry* **15**, 195
12 Buckingham, J (ed) (1982) *Dictionary of Organic Compounds* 5th edn, Vol 5, p 5009 Chapman & Hall, London