Phytochemistry, Vol 23, No 10, pp 2293–2297, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

DUNAWITHANINES A AND B, THE FIRST WITHANOLIDE GLYCOSIDES FROM *DUNALIA AUSTRALIS**

GÜNTER ADAM, NGUÊN QUYÊT CHIÊN† and NGUYÊN HÛU KHÔI‡

Institute of Plant Biochemistry, Academy of Sciences of the GDR, Halle/Saale, German Democratic Republic

(*Received* 11 *October* 1983)

Key Word Index—*Dunalia australis*, Solanaceae, withanolide glycosides, steroidal lactones, dunawithanines A and B

Abstract—Withanolide D, 7β-acetoxy-withanolide D and two new withanolide glycosides, named dunawithanines A and B, were isolated from *Dunalia australis* From physical data and chemical transformations, the structures of the new compounds were determined as (20*R*,22*R*)-*O*(3)-[2′,3′-di-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-3β,20-dihydroxy-1α-acetoxy-witha-5,24-dienolide and the corresponding *O*(3)-[β-D-glucopyranosyl(1′ → *x*)-β-D-glucopyranosyl] compound, representing the first withanolide glycosides found in the plant kingdom

INTRODUCTION

Recently we reported briefly on dunawithanines A and B from *Dunalia australis* (Griseb) Sleum [also known as *Acnistus australis* (Griseb) Griseb.] which represent the first withanolide glycosides found in the plant kingdom [1] In this paper, detailed results on the isolation and structures of both constituents are given Furthermore, besides the free withanolides which were obtained previously [2, 3] from this plant, withanolide D and 7β-acetoxywithanolide D were isolated

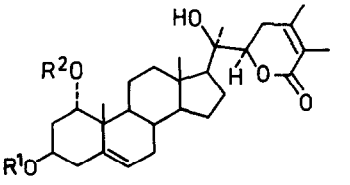
RESULTS AND DISCUSSION

Methanolic extraction of dried sprouts followed by repeated column chromatography on silica gel and alumina gave, besides withanolide D (0 003%) and 7β-acetoxy-withanolide D (0 004%), the crystalline glycosides dunawithanines A (**4**) and B (**10**) in 0 18 and 0 045% yield, respectively Acid hydrolysis of **4** or **10** (1 N methanolic HCl, 5 hr reflux) afforded in both cases D-glucose and an aglycone which was shown to be (20*R*,22*R*)-1α,3β,20-trihydroxy-witha-5,24-dienolide (**1**) by spectral data and chemical transformations [1] as well as by X-ray analysis of its monohydrate [4] Independently **1** was obtained as a free withanolide (deacetyl physalolactone B) from *Withania somnifera* chemotype III [5]

The composition $C_{48}H_{74}O_{20}\,3H_2O$ for dunawithanine A (**4**) was derived from elemental analysis Its FDMS [6] with peaks at *m/z* 1009 $[M+Na]^+$ and 516 $[M+2Na]^{2+}$ confirmed the MW of 986 Peaks due to the stepwise loss of three glucose units at *m/z* 847 $[1009-\text{glucosyl}+H]^+$, 685 $[847-\text{glycosyl}+H]^+$ and 524 $[685-\text{glucosyl}]^+$ indicated the presence of a triglucoside The typical withanolide signals in the 100 MHz ^{1}H NMR spectrum (Table 1) corresponded well with those of the 1α-monoacetyl withanolide **3** (also isolated independently as physalolactone B [7]) prepared via mild alkaline hydrolysis of the diacetate **2** Alkaline hydrolysis of **4** with 0 1 N methanolic NaOMe (72 hr at 20°) gave deacetyl dunawithanine A (**5**), which lacked ^{1}H NMR acetyl signals

Selective cleavage of the glucosidic linkage in **4** was achieved by oxidative Smith degradation [8], which gave 30% of the crystalline monoglucoside **7** The typical withanolide ^{1}H NMR signals of **7** corresponded with those of the triglucoside **4** (Table 1) A doublet at δ4 33 (*J* = 7 Hz) for the anomeric sugar proton [9, 10] indicated the β-glucosidic linkage of the remaining glucose at C-3 Alkaline hydrolysis of **7** led to deacetyl monoglucoside **8** A second Smith degradation starting from **7** yielded 33% of the monoacetate **3** which proved unequivocally the 1α-position of the acetyl function as well as the 3β-position of the sugar side chain moiety in **4** Partial acid hydrolysis of **4** (1 N methanolic HCl, 1 hr reflux) yielded aglycone **1** (54%), 1α-monoacetate **3** (6 7%), the monoglucoside **7** (2 7%) and the deacetyl monoglucoside **8** (6 6%), which were isolated by column chromatography on silica gel

For detection of the sugar sequence, the glycoside **4** was methylated with MeI–BaO–$Ba(OH)_2$ in DMFA following the procedure of Kuhn *et al* [11] Upon column



	R¹	R²
1	H	H
2	Ac	Ac
3	H	Ac

*Part of the thesis of N Q Chien, Academy of Sciences of the G D R, 1980

†Present address National Research Centre of the S R V, Institute of Chemistry, Nghia do-Tu Liem, Hanoi, S R V

‡Present address National Research Centre of the S R V, Branch Dalat, Bui Thi Yuan, Dalat, S R V

Table 1 ^{1}H NMR spectral data of dunawithanines A (4) and B (10) and derivatives (100 MHz, CD_3OD, HMDS as internal standard)

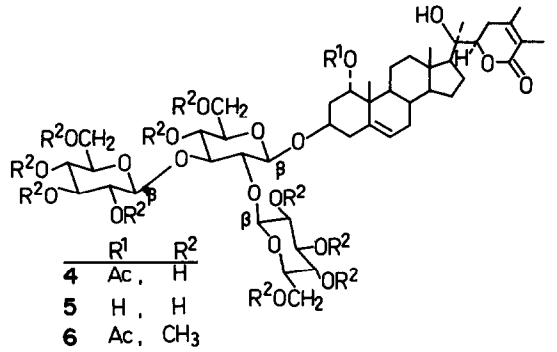
Compound	3*	4	5	6	7	10
H-1	5 05 *br s*	5 00 *br s*	†	4 92 *br s*	4 97 *br s*	4 97 *br s*
H-3	3 80 *m*	†	†	†	†	†
H-6	5 55 *d* (4)‡	5 55 *d* (5)	5 50 *d* (4)	5 37 *d* (4)	5 57 *d* (4)	5 47 *d* (4)
H-22	4 22 *dd* (12,4)	4 18 *dd* (12,4)	†	4 16 *dd* (12,4)	4 17 *dd* (12,4)	4 17 *dd* (12,4)
3H-18	0 83 *s*	0 83 *s*	0 82 *s*	0 83 *s*	0 81 *s*	0 80 *s*
3H-19	1 05 *s*	1 06 *s*	0 96 *s*	1 02 *s*	1 05 *s*	1 03 *s*
3H-21	1 25 *s*	1 22 *s*	1 22 *s*	1 22 *s*	1 20 *s*	1 19 *s*
3H-27,28	1 93 *s*	1 82 *s*	1 80 *s*	1 78 *s*	1 80 *s*	1 80 *s*
		1 84 *s*	1 84 *s*	1 87 *s*	1 93 *s*	1 92 *s*
–OAc	2 00 *s*	2 00 *s*	—	1 94 *s*	1 97 *s*	1 97 *s*
Anomeric	—	†	†	4 33 *d* (7)	4 33 *d* (7)	4 44 *d* (7)
glucose	—	†	†	4 66 *d* (7)	—	4 57 *d* (7)
protons	—	†	†	4 78 *d* (7)	—	—

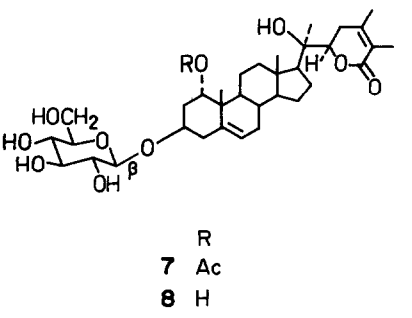
*In $CDCl_3$
†Could not be identified
‡*J* (Hz)

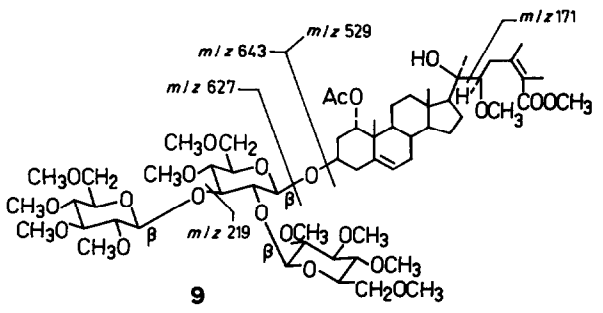
chromatography of the reaction product on silica gel, 28% deca-*O*-methyl dunawithanine A (**6**) and 22% of the lactone ring-opened dodeca-*O*-methyl derivative (**9**) were obtained

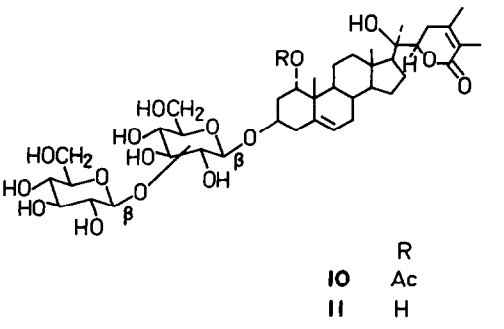
Whilst the mass spectrum of **6** upon positive ionization exhibited no molecular ion peak, under conditions of negative ionization a molecular ion at *m/z* 1127 $[M+1]^-$ was indicated besides characteristic sugar fragments at *m/z* 643 [decamethyltriglucosyl fragment including O(3)]$^-$ and *m/z* 407 [643 – tetramethylglucosyl – OH]$^-$

Compared to **6**, compound **9** upon negative ionization showed the molecular ion peak at *m/z* 1171 $[M-1]^-$









besides typical fragments of the methylated sugar part at m/z 643 and 407 (see formula **9**) Under positive ionization, the loss of m/z 171 instead of the characteristic fragment at m/z 126 of 20-hydroxylated withanolides [2] suggested hydrolysis of the δ-lactone ring with simultaneous methylation Such a structural feature was also confirmed by IR absorption at 1725 (α,β-unsaturated ester) and 1730 cm^{-1} (OAc), as well as a UV maximum at 228 nm ($\log \varepsilon = 3\,58$) (trisubstituted α,β-unsaturated ester), which is optically inactive unlike the α,β-unsaturated δ-lactone of withanolides [1, 2]

Acid hydrolysis of **6** gave, besides the aglycone **1**, two methylated sugars identified as 2,3,4,6-tetra-*O*-methyl-α-D-glucose (24%) and 4,6-di-*O*-methyl-α-D-glucose (27%) by direct comparison with authentic specimens

After these results, only the configuration of the three glucosidic linkages in **4** remained open All three were found to be β by application of Klyne's rule [12] ($[M]_D$ calculated $-11°$, found 0°) Also the three doublets at δ4 33, 4 66 and 4 78, each with $J = 7$ Hz, in the ^1H NMR spectrum of **6** indicated the $^4C_1$-conformation of the glucose [9, 10]

The structure of the minor glucoside dunawithanine B (**10**) is closely related to **4** Its FDMS with peaks at m/z 847 $[M + Na]^+$ and 435 $[M + 2Na]^{2+}$ as well as fragments at m/z 685 $[M + Na - glucosyl + H]^+$ and 524 $[M + Na - 2glucosyl + 2H]^+$ indicated an MW of 824 and the diglucosidic nature of the compound The agreement of important signals in the ^1H NMR spectrum with those of **4** and **3** (Table 1) suggested an identical substitution pattern for the withanolide part Two doublets at δ4 44 and 4 57, each with $J = 7$ Hz, for the anomeric sugar protons and application of Klyne's rule ($[M]_D$ calculated $+52°$, found $+103°$) revealed again the β-configuration of the two glucosidic linkages As with **4**, alkaline hydrolysis yielded deacetyl dunawithanine B (**11**)

On the basis of the above results, dunawithanines A and B were established as (20*R*,22*R*)-*O*(3)-[2′,3′-di-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-3β,20-dihydroxy-1α-acetoxy-witha-5,24-dienolide (**4**) and the corresponding (3)-[β-D-glucopyranosyl-(1′ → *x*)-β-D-glucopyranosyl] compound (**10**), respectively, representing the first naturally-occurring withanolide glycosides Very recently, Kirson *et al* [13] reported a third member named physalolactone B 3-*O*-β-D-glucopyranoside from *Physalis peruviana* which is structurally identical to our monoglucoside **7** prepared from **1**

EXPERIMENTAL

Mps are corrected Specific rotations in $CHCl_3$ unless otherwise stated IR spectra were determined in nujol UV and ORD were taken in MeOH Low resolution MS electron attachment mass spectrograph of the Research Institute M von Ardenne, Dresden (positive and negative ionization) High resolution EIMS Joel instrument MS D 100 FDMS Varian instrument MAT 731 CC silica gel Merck grade II, unless otherwise stated

Isolation Dried and powdered sprouts (1 8 kg) of *Dunalia australis* (grown in a greenhouse) were extracted successively with $CHCl_3$ and MeOH (each 15 l) The MeOH soln was concd to 1 l under red pres, diluted with H_2O and extracted exhaustively with C_6H_6–Et_2O (1 1) followed by $CHCl_3$–MeOH (9 1) The residue of the latter extract (1 g) was chromatographed over silica gel (Merck, grade III) The progress of the separation was followed by TLC on silica gel ($CHCl_3$–MeOH–H_2O, 70 20 3) Elution with $CHCl_3$–MeOH–H_2O (100 16 1 6) gave 0 8 g (0 045%) dunawithanine B (**10**) Crystals (MeOH–Et_2O), mp 192–195° dec, $[\alpha]_D^{25}$ +12 5° (MeOH, *c* 0 5), R_f 0 46 FDMS (emitter current 28–29 mA) m/z (rel int) 847 $[M + Na]^+$ (100), 721 $[847 - C_7H_{10}O_2]^+$ (14), 685 $[847 - glucosyl + H]^+$ (2 3), 523 $[685 - glucosyl + H]^+$ (3 2), 435 $[M + 2Na]^{2+}$ (11) IR ν_{max} cm^{-1} 1135 (α,β-unsaturated δ-lactone), 1240 (OAc), 1700 (α,β-unsaturated δ-lactone), 1730 (OAc), 3400 (OH) UV λ_{max} nm ($\log \varepsilon$) 228 (3 86) ORD $[\Theta]_{264}$ +1810°, $[\Theta]_{250}$ 0°, $[\Theta]_{232}$ −1360° ($a = +31\,7$) For ^1H NMR data see Table 1

Further elution with $CHCl_3$–MeOH–H_2O (100 16 1 6) gave 3 2 g (0 18%) dunawithanine A (**4**) Crystals (MeOH–Me_2CO), mp 208–213° dec, $[\alpha]_D^{25}$ 0° (MeOH, *c* 0 4), R_f 0 35, $C_{48}H_{74}O_{20}$ $3H_2O$ (Found C, 56 24, H, 7 87 Calc C, 56 57, H, 7 76%) FDMS (emitter current 28–30 mA) m/z (rel int) 1009 $[M + Na]^+$ (100), 847 $[1009 - glucosyl + H]^+$ (2 4), 685 $[847 - glucosyl + H]^+$ (1 8), 524 $[685 - glucosyl]^+$ (2 4), 516 $[M + 2Na]^{2+}$ (2 0) IR ν_{max} cm^{-1} 1250 (OAc), 1630 (sh), 1700 (α, β-unsaturated δ-lactone), 1730 (OAc), 3400 (OH) UV λ_{max} nm ($\log \varepsilon$) 228 (3 67) ORD $[\Theta]_{267}$ +2780°, $[\Theta]_{353}$ 0°, $[\Theta]_{230}$ −970° ($a = +37\,5$)

The residue of the C_6H_6–Et_2O extract (15 g) was chromatographed over alumina (Merck, grade II) to give upon elution with C_6H_6–Me_2CO (9 1) 60 mg (0 003%) withanolide D Crystals ($CHCl_3$–EtOAc), mp 241–249°, $[\alpha]_D^{25}$ +145° (*c* 0 2) identical to an authentic specimen

Further elution with C_6H_6–Me_2CO (9 1) gave 70 mg (0 004%) 7β-acetoxy-withanolide D Fine crystals (*n*-hexane–Me_2CO), mp 184–186°, $[\alpha]_D^{26}$ +126° (*c* 0 3), identical to an authentic specimen

Further elution with C_6H_6–Me_2CO (4 1) yielded (20*R*,22*R*)-4β,7β,20-trihydroxy-1-oxo-witha-2,5,24-trienolide and (20*R*, 22*R*)-4β,7β,20-trihydroxy-1-oxo-5β,6β-oxido-witha-2,24-dienolide, already earlier isolated from *Dunalia australis* [2, 3]

Acid hydrolysis of **4** *or* **10** **4** or **10** (300 mg) were refluxed for 5 hr with 1 N methanolic HCl (50 ml) The soln was concd under red pres and diluted with 50 ml H_2O The collected ppt was chromatographed over silica gel to give upon elution with $CHCl_3$–MeOH (97 3) 100 mg (67%) (20*R*,22*R*)-1α,3β-20-trihydroxy-witha-5,24-dienolide (**1**) Plates (Me_2CO), mp 281–284° $[\alpha]_D^{25}$ +22 2° (*c* 0 5), lit [5] mp 273°, $[\alpha]_D$ +19 8°

For detection of the sugars, the aq soln of the hydrolysis was refluxed for 3 hr, neutralized with Dowex 1 and concd under red pres Upon PC [Schleicher & Schull 2043b, *n*-BuOH–HOAc–H_2O (4 1 5) and H_2O-saturated C_6H_5OH, detection with aniline–phthalic acid], only glucose of R_f 0 17 and 0 50, respectively, was detected

Diacetate **2** Fine crystals (*n*-hexane), mp 135°, $[\alpha]_D^{25}$ +30° (*c* 0 4), lit [5] mp 85–86° (EtOAc), $[\alpha]_D$ +17 6°

O(1)-*Monoacetate* **3** Compound **2** (30 mg) in MeOH (50 ml) was stirred with Na_2CO_3 for 2 hr at room temp Evapn and chromatographic purification of the residue over silica gel yielded upon elution with *n*-hexane–Me_2CO (4 1) 15 mg (50%) **3** Plates (*n*-hexane–Me_2CO), mp 239–240°, $[\alpha]_D^{25}$ +35 6° (*c* 0 36) MS (positive ionization), 10–16 eV, m/z (rel int) 440 $[M - MeCOOH]^+$ (23), 422 $[422 - H_2O]^+$ (23), 404 $[422 - H_2O]^+$ (7), 375 $[M - 125]^+$ (15), 357 $[375 - H_2O]^+$ (20), 315 $[375 - MeCOOH]^+$ (69), 297 $[315 - H_2O]^+$ (56), 279 $[297 - H_2O]^+$ (27), 169 (64), 126 (100), 125 (78) IR ν_{max} cm^{-1} 1135, 1702 (α, β-unsaturated δ-lactone), 1250, 1730 (OAc), 3450 (OH) UV λ_{max} nm ($\log \varepsilon$) 229 (3 80) ORD $[\Theta]_{265}$ +3750°, $[\Theta]_{250}$ 0°, $[\Theta]_{235}$ −770° ($a = +35\,2$)

Deacetyl dunawithanine A (**5**) Compound **4** (200 mg) in 30 ml 0 1 N abs methanolic NaOMe was left for 72 hr at room temp Upon neutralization with dil HOAc the soln was evapd under red pres, the residue dissolved in 20 ml *n*-BuOH and washed 3 × with H_2O Evapn and crystallization gave 130 mg (65%) **5**

Crystals (MeOH–Et_2O), mp 216° dec, $[\alpha]_D^{24}$ +1,3° (*c* 0 78) IR ν_{max} cm^{-1} 1135, 1690 (α,β-unsaturated δ-lactone), 3400 (OH) UV λ_{max} nm (log ε) 228 (3 93) ORD $[\Theta]_{265}$ +1760°, $[\Theta]_{250}$ 0°, $[\Theta]_{235}$ −1820° (*a* = +35 8)

Deacetyl dunawithanine B (**11**) From **10** as described before (50%) Crystals (MeOH–EtOAc), mp 192° dec, $[\alpha]_D^{24}$ +3 4° (MeOH, *c* 0 29) IR ν_{max} cm^{-1} 1135, 1690 (α,β-unsaturated δ-lactone), 3400 (OH) UV λ_{max} nm (log ε) 229 (3 73) ORD $[\Theta]_{266}$ +1280°, $[\Theta]_{252}$ 0°, $[\Theta]_{235}$ −1280° (*a* = +25 6)

Oxidative Smith degradation [8] *of dunawithanine A* (**4**) *to* **7** Compound **4** (500 g) in MeOH (100 ml) was stirred with $NaIO_4$ (1 5 g) in H_2O (15 ml) for 16 hr at 4° After standing for 12 hr at room temp and filtration, the soln was concd under red pres and *n*-BuOH (100 ml) was added The organic phase was washed with H_2O, evapd, and the residue obtained dissolved in MeOH (100 ml) After addition of $NaBH_4$ (1 g), the soln was stirred for 1 hr at room temp and neutralized with 5% HOAc in MeOH 1 N methanolic HCl (25 ml) was added and the soln left for 24 hr at room temp Evapn under red pres, addition of 100 ml *n*-BuOH, repeated washing with H_2O, and evapn gave a residue which was chromatographed on 8 g silica gel Upon elution with $CHCl_3$–MeOH (49 4), 100 mg (30%) monoglucoside **7** was obtained Crystals (MeOH–H_2O), mp 172–175° dec, $[\alpha]_D^{25}$ +15 2° (MeOH, *c* 0 4) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1135, 1690 (α,β-unsaturated δ-lactone), 1255, 1730 (OAc), 3450 (OH) UV λ_{max} nm (log ε) 230 (3 87) ORD $[\Theta]_{272}$ +725°, $[\Theta]_{240}$ −3015° (*a* = +37 3), lit [13] amorphous, $[\alpha]_D$ +3 7° (pyridine)

Oxidative Smith degradation of monoglucoside **7** *to* **3** From **7** (60 mg) as described before ($CHCl_3$ instead of *n*-BuOH was used for the extraction after acid hydrolysis) Elution of the silica gel column with *n*-hexane–Me_2CO (8 3) gave 15 mg (33%) **3**, mp 236–239°, identical in every aspect to *O*(1)-monoacetate **3** prepared from **1** via diacetate **2**

Deacetyl monoglucoside **8** Compound **10** (10 mg) in 0 1 N methanolic NaOMe (2 ml) was left for 48 hr at room temp After neutralization with dil HOAc, evapn under red pres, and addition of $CHCl_3$–MeOH (9 1), the soln was dried over Na_2SO_4 and evapd to give 5 mg (50%) **8** Crystals (EtOAc), mp 178–182°, $[\alpha]_D^{26}$ +26 1° (MeOH, *c* 0 3) MS (positive ionization), 10–16 eV, *m/z* (rel int) 584 $[M-2H_2O]^+$ (24), 440 [M − glucose]$^+$ (34), 422 $[440-H_2O]^+$ (100), 404 $[422-H_2O]^+$ (74), 333 [M − glucose − 125]$^+$ (33), 315 $[333-H_2O]^+$ (59), 297 $[315-H_2O]^+$ (61) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1135, 1700 (α,β-unsaturated δ-lactone) 3400 (OH) UV λ_{max} nm (log ε) 229 (3 86)

Partial acid hydrolysis of **4** Compound **4** (600 mg) was refluxed with 1 N methanolic HCl (100 ml) for 1 hr The soln was evapd under red pres, the residue was dissolved in *n*-BuOH (100 ml), washed with H_2O, evapd under red pres, and the residue obtained (300 mg) chromatographed over silica gel (15 mg) Elution with $CHCl_3$–MeOH gradients gave 20 mg *O*(1)-monoacetate **3** (49 1), 150 mg withanolide **1** (97 3), 11 mg monoglucoside **7** (9 1) and 25 mg deacetyl monoglucoside **8** (4 1), identical in every aspect to the otherwise prepared specimen

Methylation of dunawithanine A (**4**) *to* **6** *and* **9** Compound **4** (600 mg) was shaken in DMF (12 ml) with BaO (2 4 g), $Ba(OH)_2$ $8H_2O$ (48 mg) and MeI (2 6 ml) [11] for 24 hr, and after addition of further MeI (1 ml) and BaO (100 mg), again for 24 hr at room temp The soln was diluted with $CHCl_3$ (200 ml) and after filtration, extraction with H_2O and aq $Na_2S_2O_3$ evapd CC of the residue (626 mg) over silica gel (Woelm, 30 g) gave upon elution with *n*-hexane–$CHCl_3$ (1 4) 200 mg (28%) deca-*O*-methyl derivative **9** Scales (*n*-hexane–$CHCl_3$), mp 76–80°, $[\alpha]_D^{25}$ +3 5° (*c* 0 5) MS (negative ionization), 10–16 eV, *m/z* (rel int) 1171 $[M-1]^-$ (48), 999 $[M-1-171]^-$ (26), 643 (deca-methyl triglucosyl fragment) (46), 545 $[M-627]^-$ (26), 527 $[545-H_2O]^-$ (70), 407 [643 − tetramethyl glucosyl − OH]$^-$ (96), 333 (100), 331 (100) MS (positive ionization), 10–16 eV, *m/z* (rel int) 643 (decamethyl triglucosyl fragment) (20), 627 (37), 529 (97), 469 $[529-HOAc]^+$ (78), 357 $[529-1-171]^+$ (95), 297 $[357-HOAc]^+$ (96), 279 $[297-H_2O]^+$ (83), 219 (tetramethyl glucosyl fragment) (100), 187 $[219-MeOH]^+$ (91) IR ν_{max} cm^{-1} 1240, 1730 (OAc), 1725 (α,β-unsaturated ester), 3500 (OH) UV λ_{max} nm (log ε) 228 (3 58)

Elution with $CHCl_3$ gave 150 mg (22%) deca-*O*-methyl-dunawithanine A (**6**), scales (*n*-hexane–$CHCl_3$), mp 92–95°, $[\alpha]_D^{26}$ +5 1° (*c* 0 4) MS (negative ionization), 10–16 eV, *m/z* (rel int) 1127 $[M+1]^-$ (12), 1108 $[M-H_2O]^-$ (53), 1048 [1108 − HOAc]$^-$ (6), 889 $[1108-219]^-$ (7), 643 (decamethyl triglucosyl fragment) (16), 481 (27), 463 $[481-H_2O]^-$ (55), 421 [481 − HOAc]$^-$ (33), 407 [643-tetramethyl glucosyl − OH]$^-$ (76) MS (positive ionization), 10–16 eV, *m/z* (rel int) 499 [M − 627]$^+$ (5), 485 (46), 465 $[M-643-H_2O]^+$ (56), 439 [499 − HOAc]$^+$ (36), 421 $[439-H_2O]^+$ (49), 405 $[465-HOAc]^+$ (46), 391 (81), 357 $[M-643-126]^+$ (43), 297 $[357-HOAc]^+$ (44), 219 (tetramethyl glucosyl fragment) (85), 187 [219 − MeOH]$^+$ (100) IR ν_{max} cm^{-1} 1240, 1730 (OAc), 1700 (α,β-unsaturated δ-lactone), 3500 (OH) UV ν_{max} nm (log ε) 228 (3 69) ORD $[\Theta]_{266}$ +1940°, $[\Theta]_{250}$ 0°, $[\Theta]_{233}$ −2200° (*a* = +41 4)

Acid hydrolysis of **6** Compound **6** (200 mg) was refluxed with 1 N methanolic HCl (50 ml) for 5 hr Upon evapn under red pres and dilution with H_2O (50 ml), the ppt was crystallized to give **1** The aq filtrate was refluxed for 3 hr and extracted 5× with $CHCl_3$ The aq soln was neutralized with Dowex 1 and evapd CC of the residue of the $CHCl_3$ extract over 2 g silica gel gave upon elution with C_6H_6–Me_2CO (7 3) 20 mg (24%) 2,3,4,6-tetra-*O*-methyl-α-D-glucose Needles (*n*-hexane), mp 88–91°, $[\alpha]_D^{25}$ +87 5 → 83 0° (H_2O, *c* 0 2), identical in every aspect to an authentic [14] sample CC of the residue of the aq soln over 2 g silica gel gave upon elution with $CHCl_3$–MeOH (47 3) 10 mg (27%) 4,6-di-*O*-methyl-α-D-glucose Needles (MeOH–$CHCl_3$), mp 165–169°, $[\alpha]_D^{25}$ +76 9 → 62 9° (H_2O, *c* 0 14), identical in every aspect to an authentic sample prepared from α-tomatine [15]

Acknowledgements—We are indebted to Prof H R Schulten, Wiesbaden, for the FD-mass spectra and to Dr W Schade, Jena, for the high-resolution mass spectra We thank Prof D Lavie, Rehovot, for the authentic samples of withanolide D, and 7β-acetoxy-withanolide D, and Dr H Ripperger, Halle, for 2,3,4,6-tetra-*O*-methyl-D-glucose

REFERENCES

1 Adam, G, Chien, N Q and Khoi, N H (1981) *Naturwissenschaften* **67**, 425
2 Adam, G and Hesse, M (1972) *Tetrahedron* **28**, 3527
3 Kirson, I, Lavie, D, Albonico, S M and Juliani, H R (1970) *Tetrahedron* **26**, 5063
4 Reck, G, Khoi, N H and Adam, G (1982) *Cryst Struct Commun* **11**, 355
5 Vande Velde, V and Lavie, D (1981) *Phytochemistry* **20**, 1359
6 For the method see Schulten, H-R, Komori, T and Kawasaki, T (1977) *Tetrahedron* **33**, 2595
7 Ray, A B, Ali, A, Sahai, M, Schiff P L, Jr, Knapp, J E and Slatkin, D J (1981) *Chem Ind* 62
8 Lewis, B A, Cyr, M J St and Smith, F (1968) *J Org Chem* **33**, 3139
9 Heguchi, R, Miyahara, K and Kawasaki, T (1972) *Chem Pharm Bull (Tokyo)* **20**, 1935

10 Coxon, B (1972) *Adv Carbohydr Chem Biochem* **27**, 7
11 Kuhn, R, Egge, H, Brossmer, G, Gaube, A, Klesse, P, Lochinger, W, Rohm, E, Trischmann, H and Tschampel, D (1960) *Angew Chem* **72**, 805
12 Klyne, W (1950) *Biochem J* **47**, 41
13 Kirson, I, Glotter, E, Ray, A B, Ali, A, Gottlieb, H E and Sahai, M (1983) *J Chem Research (S)* 120
14 Ripperger, H and Seifert, K (1975) *Tetrahedron* **31**, 1561
15 Kuhn, R, Low, I and Trischmann, H (1957) *Chem Ber* **90**, 203