Phytochemistry, Vol 27, No 5, pp 1451–1458, 1988
Printed in Great Britain

0031 9422/88 $3.00 + 0.00
Pergamon Press plc

STRUCTURAL REVISION OF PREGNANE ESTER GLYCOSIDES FROM CONDURANGO CORTEX AND NEW COMPOUNDS

S BERGER, P. JUNIOR*† and L. KOPANSKI‡

Fachbereich Chemie, Philipps-Universität, D-3550 Marburg/Lahn, F.R.G. †Institut für Pharmazeutische Biologie der Universität Düsseldorf, D-4000 Düsseldorf 1, F.R.G., ‡Schaper und Brümmer GmbH & Co. KG, D-3320 Salzgitter 61, F.R.G.

(*Received* 12 *June* 1987)

Key Word Index—*Marsdenia cundurango*; Asclepiadaceae; pregnane ester glycosides; new condurangoglycosides E, E_0, E_2 and E_3; structural revision of known condurangoglycosides.

Abstract—The pregnane ester glycoside fraction from Condurango cortex, dried bark of *Marsdenia cundurango*, has been reinvestigated. Four as yet unknown glycosides of the new aglycone condurangogenin E have been isolated by column chromatography and subsequent HPLC, and named condurangoglycoside (CG) E, E_0, E_2 and E_3. The structures of these compounds were established by combination of degradation of sugar chains and spectroscopic means (^{1}H, ^{13}C NMR, FD-MS). In addition, reinvestigation of the known pregnane ester compounds condurangogenin A, CG A, A_0 and C by selective proton-decoupling technique in gated decoupled ^{13}C NMR spectra requires the structural revision of all these and related compounds previously found in Condurango cortex. Aglycones of A, B, C, D and E series thus are esterified with acetic acid at the 11α-hydroxy group and with cinnamic acid at the 12β-hydroxy group of the steroid skeleton.

INTRODUCTION

Marsdenia cundurango Rchb. f., an Asclepiadaceae plant, is native to the north-western part of South America (Ecuador, Peru, Columbia). The bark of this plant, Condurango cortex, used as a bitter aromatic stomachic has been previously investigated by Tschesche *et al.* [1–4] and Mitsuhashi *et al.* [5, 6]. These investigations led to the isolation and identification of several polyhydroxy pregnane ester genins and glycosides named condurangogenin A (**11**) and C, CG A (**7**), C (**9**) (trisaccharides), A_1, C_1 (pentasaccharides) [1–4], CG A_0 (**8**), C_0 (tetrasaccharides), condurangogenin B and CG B_0, D_0 (**10**), 20-*O*-methylcondurangoglycoside D_0 and 20-*iso*-*O*-methylcondurangoglycoside D_0 (tetrasaccharides) [5, 6]. Finally, Pailer and Ganzinger [7] reported the isolation and structure elucidation of the two basic compounds condurangamin A and B. Very similar constituents have been isolated from leaves of *Marsdenia erecta* R. Br. and stems of *Marsdenia tenacissima* (Roth) Wight and Arn. by Reichstein *et al.* [8] and Mitsuhashi *et al.* [9], respectively. In both cases, the order of acyl substituents in diester linkages at C-11 and C-12 remained unsettled.

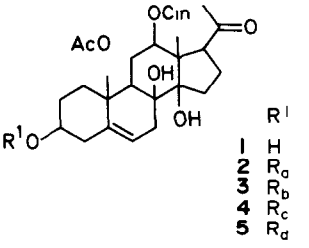
We now wish to report the structures of four glycosides of the new aglycone condurangogenin E (**1**) (11α-acetoxy-12β-cinnamoyloxy-3β, 8β, 14β-trihydroxy-pregn-5-ene-20-one), isolated from commercially available Condurango cortex. In addition, the structures of already known aglycones from A–D series and related glycosides have been revised.

RESULTS AND DISCUSSION

The chloroform-soluble fraction of a crude extract from Condurango cortex (see Experimental) was subjected to column chromatography and subsequent HPLC to give the eight condurango glycosides (CG) E (**2**), E_0 (**3**), E_2 (**4**) and E_3 (**5**) as well as CG A (**7**) A_0 (**8**), C (**9**) and D_0 (**10**). All these glycosides exhibited a positive Xanthydrol reaction, thus indicating the presence of 2-deoxy sugars in the molecules.

Mild acidic hydrolysis of CG E (**2**) ($C_{53}H_{76}O_{18}$) afforded **1**, cymarose and pachybiose. Stronger conditions led to cymarose, oleandrose and 6-deoxy-3-*O*-methylallose, identical with authentic samples by TLC comparison. Both dideoxy pyranoses possess only two free hydroxy functions (C-1, C-4), and therefore the sugar sequence must be linear. Compound **1**, after spraying with vanillin–sulphuric acid reagent, gave a blue colour, and it was not identical with one of the condurangogenins already known.

The ^{1}H NMR spectrum of **2** showed signals due to each of the three secondary methyl groups and in addition three methoxy groups of the sugar moieties. The coupling constants of the three anomeric protons proved β-glycosidic linkages. ^{13}C NMR spectral data (Table 3) are in excellent agreement with those reported for the 3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-olean-



*Author to whom correspondence should be addressed

Table 1 ^{13}C NMR shifts of compounds **2**–**5** (aglycone part), **1** and cynanchogenin (**6**) (data taken from [10]) in C_5D_5N

C	2	3	4	5	1	6
1	39 92	39 96	39 94	39 91	39 31	39 2
2	30 22	30 21	30 23	30 20	32 73	31 9
3	77 85	77 83	77 82	77 83	71 59	71 5
4	39 34	39 36	39 36	39 32	43 89	43 1
5	139 75	139 85	139 77	139 71	140 63	140 2
6	118 76	118 65	118 77	118 79	118 12	118 4
7	36 92	36 98	36.93	36 89	36 92	34 1
8	76 06	76 07	76 08	76 04	76 15	74 5
9	49 20	49 25	49 20	49 18	49 22	44 7
10	40 60	40 61	40 61	40 58	40 86	37 4
11	71 79	71 77	71 80	71 78	71 86	25 0
12	78 71	78 67	78 73	78 71	78 77	72 3
13	55 64	55 63	55 67	55 64	55 68	55 6
14	85 62	85 63	85 64	85 61	85 66	87 4
15	35 60	35 63	35 62	35 38	35 61	35 1
16	24 41	24 43	24 43	24 40	24 40	21 7
17	59 41	59 38	59 43	59 41	59 47	60 5
18	13 61	13 55	13 64	13 62	13 66	15 8
19	18 16	18 15	18 18	18 16	18 31	18 3
20	213 60	213 72	213 63	213 53	213 52	209 0
21	31 60	31 62	31 62	31 57	31 57	32 0
1′	170 00	169 94	170 05	170 01	170 10	
2′	21 55	21 51	21 58	21 55	21 63	
1″	167 20	167 14	167 22	167 20	167 27	166 0
2″	146 50	146 46	146 53	146 50	146 55	114 1
3″	118 16	118 19	118 17	118 14	118 17	165 1
4″	134 82	134 86	134 84	134 80	134 82	38 0
5″	129 36	129 33	129 39	129 36	129 29	20 9
6″	128 81	128 77	128 84	128 83	128 85	20 9
7″	130 96	130 90	130 99	130 97	131 00	16 4

dropyranosyl-(1→4)-β-D-cymaropyranosyl unit of CG A and C [5]

The aglycone part of **2** revealed signals due to a Δ^5-double bond and a further hydroxy group besides those for C-3 involved in the glycosidic bond, C-11 and C-12 esterified with acetic and cinnamic acid, respectively, and C-14 with the free hydroxy group Comparison with spectra of known compounds such as cynanchogenin (17α) [10] clearly indicated 17β-configuration for condurangogenin E The ^{1}H NMR spectral data confirmed the structure of this new genin as an 11, 12-*O*-acetylcinnamoyl-derivative of 17β-marsdenin [11], disregarding the order of acyl substituents in ester linkages Thus, the structure of CG E (**2**) was deduced to be condurangogenin E 3-*O*-β-D-6-deoxy-3-*O*-methyl-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside

CG E_0 (**3**) ($C_{59}H_{86}O_{23}$) was one of the most polar compounds of the investigated fraction Due to its hygroscopic behaviour, the ^{1}H NMR spectrum was not resolved sufficiently (see Experimental) but suggested the presence of four sugar moieties in the molecule The ^{13}C NMR spectrum confirmed this suggestion, indicating the same sugar chain as in CG A (**7**) (Tables 2 and 3) and CG C_0 [5] Thus, the structure of **3** was given as condurangogenin E 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-6-deoxy-3-*O*-methyl-allopyranosyl-(1→4)-β-D-cymaropyranoside

CG E_2 (**4**) ($C_{60}H_{88}O_{21}$) showed a pattern in its ^{1}H NMR spectrum very similar to that of **2** except for the signals of the secondary methyl functions, methoxy groups and anomeric protons, indicating one further 3-*O*-methyl-dideoxy unit in β-glycosidic linkage in the sugar chain The ^{13}C NMR spectrum of **4** revealed signals due to two β-D-cymarose units and for β-D-oleandrose and 3-*O*-methyl-β-D-6-deoxyallose moieties, in that order, from the aglycone part This conclusion was proved by acidic cleavage of the glycosidic bonds

In the case of mild hydrolysis, compound **4** afforded **1** as well as cymarose and pachybiose, identified by TLC comparison However, stronger hydrolysis conditions led to the identification of cymarose, oleandrose and 3-*O*-methyl-6-deoxyallose. On spraying with Xanthydrol reagent, the cymarose spot appeared about twice as strong as that of oleandrose The structure of CG E_2 (**4**) thus was deduced to be condurangogenin E 3-*O*-β-D-6-deoxy-3-*O*-methyl-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside

CG E_3 (**5**) ($C_{66}H_{98}O_{26}$) in the case of mild acidic hydrolysis revealed cymarose and more polar spots Stronger conditions gave cymarose, oleandrose (ratio

Table 2 ^{13}C NMR shifts of CG A (**7**), CG A_0 (**8**), CG C (**9**), CG D_0 (**10**) (aglycone part), condurangogenin A (**11**), drevogenin D (**12**) and drevogenin-D-3,12,20-triacetate (**13**) (C_5D_5N, except **11** and **13** in $CDCl_3$)

C	7	8	9	10	11	12	13*
1	37 34	37.37	37 35	37 67	37 6	40 17	38 35
2	30 48	30.51	30 52	30.51	31 8	32 91	27 63
3	76.20	76.25	76 30	76.49	70 8	71 77	73 71
4	35 57	35.61	35.63	35 62	38 5	44 09	38 83
5	44 76	44.81	44 87	44 70	44 8	141 73	139 04
6	29.41	29.44	29 60	28 52	28 9	121.79	122 08
7	28 44	28.46	28 49	29 38	28 0	28.40	27.21
8	40.02	40.05	40 15	42 12	39 2	38 41	37 65
9	50 21	50 27	50 49	49 73	50 0	50 14	49 73
10	38 01	38.04	38 14	37 93	37 6	39 80	38 69
11	71 77	71 81	72.06	72.05	72.0	71.84	70 36
12	78.51	79 54	79.86	76.85	78 3	80 67	81 52
13	54 87	54 90	54 05	65 27	54 3	54 22	52 00
14	83.99	83 97	83 98	82 03	83 8	84 58	84 92
15	33 97	34 02	33 17	26 60	34 1	34 25	32 51
16	24 37	24.41	26 80	38 58	24.6	27 23	24.87
17	58 38	58.41	52 92	58.48	57 2	54 81	50 21
18	12.44	12.47	12 51	64.58	12 3	10 07	11 66
19	11.71	11.71	12 53	12 19	10 8	19 28	18 48
20	213 73	213.81	70 41	104.01	216 4	70 62	73 13
21	31.86	31.86	23 71	20.09	33 1	23 74	18 90
1′	170 45	170.46	170 47	170.35	170 3		
2′	21.54	21.56	21 64	20.09	21 2		
1″	167.07	167.09	167 13	167.14	166 9		
2″	146 45	146.47	146 66	146.49	146 6		
3″	118 09	118 12	118 79	118.13	116 8		
4″	134 79	134 82	134.95	134.77	134 0		
5″	129 38	129 40	129.28	129 37	129 5		
6″	128 83	128 83	128 68	128 83	128 7		
7″	131 00	131 01	130 71	130 97	130 8		

*Acetyl 21 06, 21 24, 21.44, 170 13; 170 44, 172 33

2:1) and a disaccharide identified by TLC In addition, enzymatic cleavage of the remaining glycosidic bond with β-glucosidase led to the identification of glucose and 3-*O*-methyl-6-deoxyallose. These results, in combination with the ^{1}H NMR spectral data (5 anomeric protons, four secondary methyls and four methoxy groups), led to the conclusion that this sugar chain must consist of two cymarose units and one each of oleandrose, 3-*O*-methyl-6-deoxyallose and glucose moieties in that order from the aglycone site.

The ^{13}C NMR spectrum of **5** was in full agreement with the proposed structure (Tables 1 and 3). The expected glycosidation shift pattern for the terminal β-D-6-deoxy-3-*O*-methylallopyranose unit in **4** in comparison with **5** was observed for C-4 and C-5 (downfield 8 6 and upfield 1 5 ppm, respectively) in accordance with spectral data of CG A_0 (**8**) and CG C_0, reported by Mitsuhashi *et al* [5] and confirmed by our own measurements. Therefore, the structure of CG E_3 (**5**) was deduced to be condurangogenin E 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-6-deoxy-3-*O*-methyl-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Besides these novel pregnane ester glycosides of the new aglycone condurangogenin E (**1**), the known compounds CG A (**7**), A_0 (**8**), C (**9**) and D_0 (**10**) have been isolated in the course of our investigations The structures have been proven by physico-chemical data, especially the ^{13}C NMR spectra. The data given by Mitsuhashi *et al* [5, 6] are in excellent agreement with our own measurements The order of acyl substituents in diester linkage at C-11 and C-12 has been determined by Tschesche *et al.* [3] Condurangogenin A and C thus should be characterized both by 11α-cinnamoyloxy- and 12β-acetoxy groups. The structures of condurangogenin B and D have been deduced by Mitsuhashi *et al.* [5, 6] by transformation of condurangogenin C-3-monoacetate into condurangogenin B-acetate and condurangogenin A-acetate

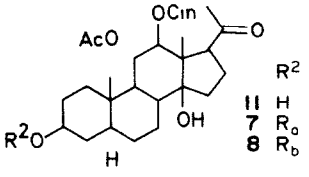
The conversion of condurangogenin E into one of the aglycones already known seemed to be difficult However, we succeeded in determining the substitution pattern by spectroscopic means

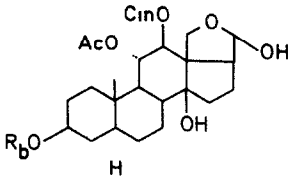
To distinguish between the two possible partial structures I and II it was necessary to correlate the ^{13}C resonance positions of the two carbonyl groups with the ^{1}H NMR resonance positions of the proton signals at C-11 and C-12, respectively.

The assignment of the proton signals is unequivocal, since the proton at C-11 forms a triplet due to spin

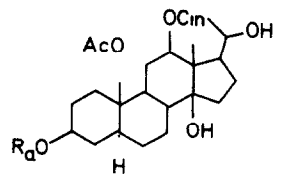
Table 3 ^{13}C NMR shifts for sugar carbons of compounds **2–5, 7–10**

C		2	3	4	5	7	8	9	10
1 Cy	1	96 55	96 57	96 56	96.56	96 14	96 22	96 15	96 18
	2	37 31	37 28	37 33	37 30	37 58	37 36	37.58	37 64
	3	77 90	77 89	77 83	77 85	77 92	77 95	77.94	77 94
	4	82 77	82 88	82 68	82 89	82 81	82 92	82.80	82.88
	5	68 97	68 96	69 07	69 10	68 94	68 99	68 94	68 96
	6	18.69	18 67	18 55	18 52	18 71	18 31	18 62	18 31
–OMe		58 82	58 81	58 91	58 95	58 84	58 87	58 84	58 85
2 Cy	1			100 41	100 46				
	2			37 14	37.11				
	3			78 08	78 08				
	4			83 20	83 19				
	5			68 96	68 96				
	6			18 61	18 66				
–OMe				58 81	58.86				
O1	1	101.88	101 80	101 78	101 82	101 93	101.83	101 90	101 80
	2	37 57	37 63	37 50	37 63	38 13	37 76	38 04	38 16
	3	79 31	79 35	79 32	79 38	79 30	79 39	79 30	79 39
	4	83 47	83 46	83 57	83 40	83 94	83 51	83 52	83 28
	5	72 07	72 01	72.12	72 07	72 05	72 05	72 06	72 06
	6	18 60	18 28	18 48	18.31	18 62	18 74	18 72	18 72
–OMe		57 13	57 32	57 05	57 34	57 17	57 35	57 16	57 31
A1	1	101 88	101 87	101 78	101 82	101 89	101 91	101 90	101 89
	2	73 28	72 68	73 30	72 71	73 28	72 70	73 28	72 69
	3	83 69	83 36	83 89	83 40	83 50	83 40	83 57	83 39
	4	74 61	83 13	74 60	83 16	74 60	83 16	74 61	83 14
	5	71 05	69 54	71 07	69 58	71 03	69 58	71 04	69.56
	6	18 94	18 88	18 92	18 91	18 93	18 92	18 93	18 90
–OMe		62 05	61 72	61 99	61 73	62 06	61 74	62 06	61 71
G1	1		106 53		106 53		106 56		106 54
	2		75 50		75 53		75 53		75 51
	3		78 32		78.33		78 35		78 31
	4		72 01		72 07		72.05		72 06
	5		78 40		78 42		78.42		78 41
	6		63 11		63 15		63 14		63 15





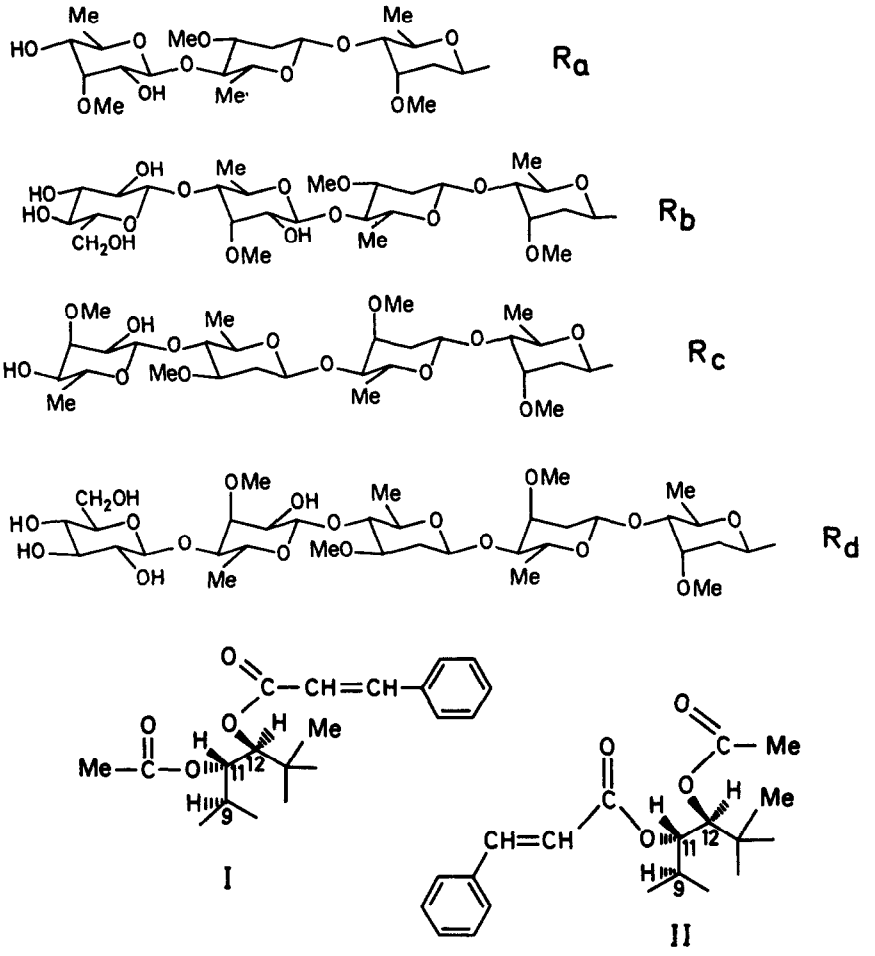
10



9

coupling both to the proton at C-12 and to that at C-9, whereas the proton at C-12 forms only a doublet

Both of the signals of the carbonyl groups form complicated multiplets in the gated decoupled ^{13}C NMR spectrum due to spin coupling with the protons within the acetate or cinnamoyl residue and due to spin coupling to protons within the steroid skeleton, where the $^{3}J_{C,H}$ coupling with the proton at the attached site will be predominant The normal gated decoupling spectrum is shown in trace A of Fig 1 for authentic condurangogenin A (**11**)



In the 400 MHz ^{1}H NMR spectrum, the signals of the protons at C-11 and C-12 are sufficiently apart to allow selective decoupling during the gated decoupling experiment. The decoupler of the instrument was therefore switched from high power broad band decoupling during the pulse delay to selective low power decoupling during the acquisition time. The result is shown in traces B and C of Fig. 1.

Removal of the coupling to the proton at C-11 reveals a quadruplet for the attached carbonyl signal, whereas removal of the coupling to the proton at C-12 reveals a doublet of doublets as the remaining signal for the other carbonyl group.

These results unequivocally demonstrate the partial structure I to be correct. In addition to these findings, it should be mentioned that the order of the chemical shifts of the carbonyl groups assigned in this manner is in accordance with the expectation, since α,β-unsaturated ester groups absorb at higher field. The experiment was repeated with the same result for the other structures shown in this paper. Thus, all aglycones of condurango glycosides are 11α-acetoxy, 12β-cinnamoyloxy pregnanes and therefore all structures of the A–D series must be revised.

The structure of condurangogenin C [3] was derived by selective acylation of drevogenin D (**12**) [11]. Acetylation and hydrogenation led to the 3,12,20-triacetyl-5α-pregnane. Subsequent cinnamoylation under strong conditions afforded a compound not distinguishable from condurangogenin C by TLC and IR spectra. We tried to reproduce this selective acylation but we did not succeed in cinnamoylation of the triacetyl-5α-pregnane However, the spectral data of drevogenin D 3,12,20-triacetate (**13**) clearly indicated one free hydroxy group at C-11 of the steroid skeleton. It might be possible that a migration of acyl substituents has taken place under conditions chosen by Tschesche and coworkers [3]. On the other hand, a certain differentiation between both structures by TLC and IR spectral data seems to be difficult.

EXPERIMENTAL

Mps. uncorr Optical rotations were measured at room temp. UV spectra were taken MeOH. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were run in $CDCl_3$ and C_5D_5N soln with TMS as int standard. CC with Baker 0·05–0.2 and 0·04–0.063 mm silica gel using either open glass columns or a Prep 10 Yvon Jobin steel column. HPLC was done using Hypersil ODS, 5 μm in a 4.0 × 250 mm column (anal mode) and a 32 × 250 mm column (prep. mode) with a mixture of MeCN–H_2O in Knauer isocratic HPLC systems at 280 nm The flow rate was 0.8–1 ml/min (anal mode) and 3.0–8.0 ml/min (prep. mode).

Commercially available extract has been used for the separation and preparative isolation of the described glycosides as

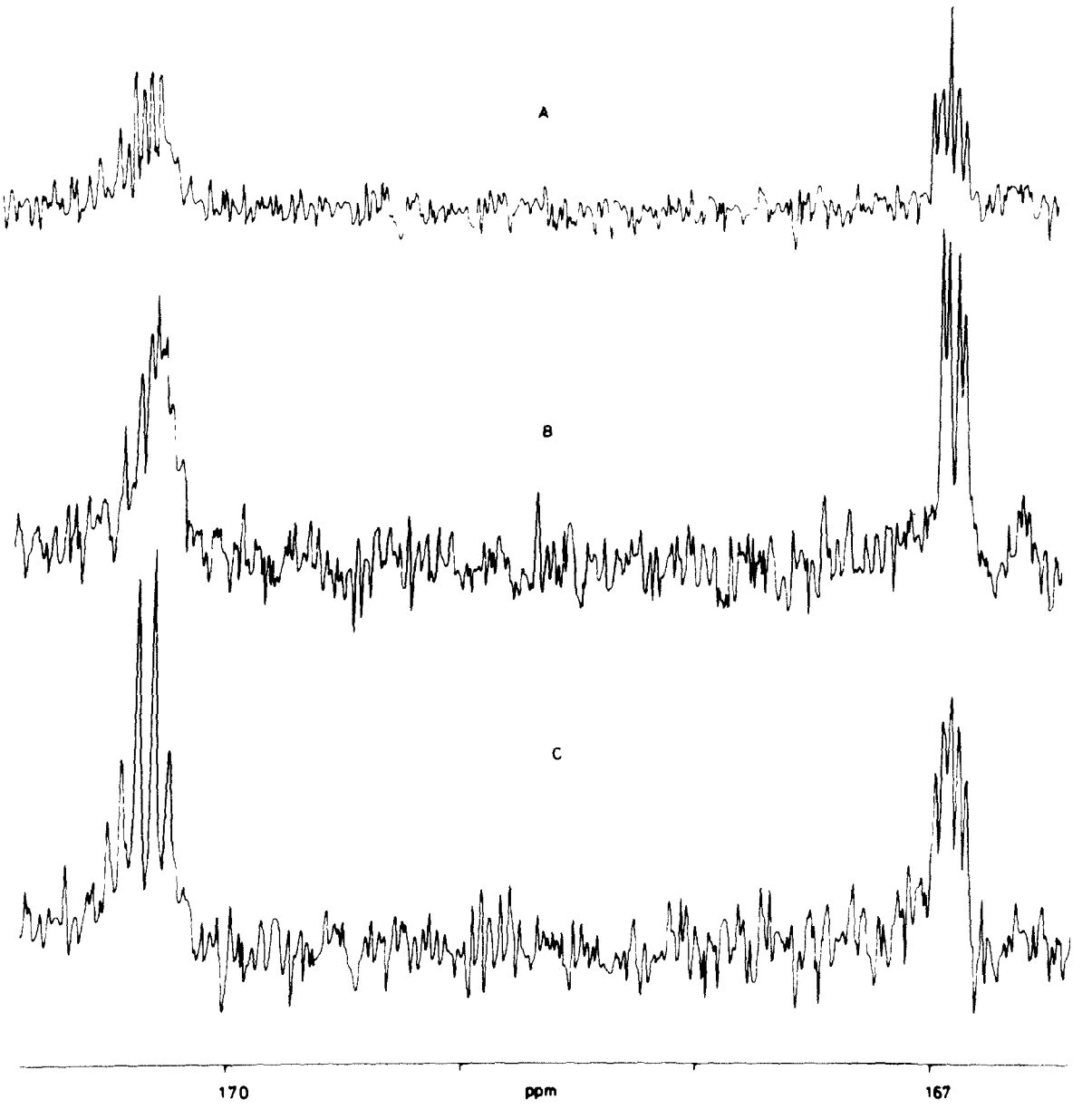


Fig 1 ^{13}C NMR spectra of compound **11** (only ester carbonyl groups shown), trace A normal gated decoupling, traces B and C selective decoupling of H-12 and H-11 protons

described in ref [3], to obtain CGA and CGC as well as condurangogenins A and C Results have been checked by us by comparison of HPLC-separation of self prepared extracts from commercially available Condurango Cortex and the profiles of chromatograms were identical Comparison with an extract from a Columbian source, provided by H Schumacher (Degussa Pharma, Sao Paolo, Brazil) led to the same result The identity of the drugs was confirmed by distinctive microscopical markers known for Condurango Cortex

Isolation of the condurangoglycosides **4**, **7**, **2** and **9**. Commercially available, dried extract of Condurango cortex (Finzelberg, West Germany, batch 1521202) (3 5 kg) was digested with $CHCl_3$, and the raw glycosides (505 g) were separated further with Et_2O into Et_2O soluble (284 g) and Et_2O insoluble glycosides (218 g)

CC of 166 g of the Et_2O soluble portion on silica gel with C_6H_5Me–$CHCl_3$–Me_2CO (0 0, 8 0, 16 0% Me_2CO) yielded two main fractions (9–12, 46 1 g and 13–15, 90.6 g)

After HPLC analysis, the compounds **4**, **7** and **2** were highly concentrated in fractions 9–12, whereas **9** was the main substance of fractions 13–15 The isolation of the pure compounds was done by twofold prep HPLC on RP silica gel with MeCN–H_2O For the first separations, samples of 300–400 mg of the preconcentrated glycosides in 2 0 ml solvent were injected, yielding 10–30 mg of highly concentrated glycosides from each run The second HPLC procedure was effected with 45- 100 mg

samples, yielding about 70% of chromatographically pure material, according to HPLC analysis. After evapn of MeCN, the pure glycosides were obtained as white, voluminous, amorphous material after lyophilization Due to their chromatographic behaviour on RP silica gel, the compounds are summarized in order of increasing polarity.

Condurangoglycoside E_2 (**4**) White amorphous material, mp 139–142°, $[\alpha]_D$ +81 5° ($CHCl_3$, *c*1 0); (Found. C, 61.50 H; 8.07. $C_{60}^{*}H_{88}O_{21}$ 2 H_2O requires: C, 61 00; H, 8 07%), IR ν_{max}^{KBr} cm^{-1} 3450, 1740, 1708, 1690, 1630, 1445, 1360, 1265, 1250, 1225, 1160, 1100–1055, 910, 860, 765, 702, FDMS *m/z* 1167 $[M+Na]^+$; UV λ_{max}^{MeOH} nm. 216, 221 and 278 (logε 4 40, 4 35 and 4 60); ^1H NMR ($CDCl_3$): δ1 20 (3H, *s*, H_3-19), 1.28 (3H, *s*, H_3-18), 1 17, 1 18, 1 22, 1 32 (3H each, *d*, *J* = 6.2, 6 2, 6.2 and 5.4 Hz), 1.87 (3H, *s*, Ac), 2 13 (3H, *s*, H_3-21), 3.05 (1H, *m*, H-17α), 3 35 (3H, *s*, OMe), 3 40 (6H, *s*, OMe), 3 62 (3H, *s*, OMe), 4.45 (1H, *dd*, *J* = 1.6, 10 Hz, anomeric), 4 72 (1H, *dd*, *J* = 1 8, about 10 Hz, anomeric overlapped), 4 75 (1H, *d*, *J* = 8 2 Hz, anomeric), 4.81 (1H, *dd*, *J* = 1 8, 10 Hz, anomeric), 4 97 (1H, *d*, *J* = 10 Hz, H-12α), 5 35 (1H, *m*, H-6), 5 80 (1H, *t*, *J* = 10 Hz, H-11β), 6 44, 7 75 (1H each, *J* = 16 Hz AB), 7 39, 7.55 (3H, 2H, arom)

Condurangoglycoside A (**7**). White amorphous material, mp 131–136°, $[\alpha]_D$ +66° ($CHCl_3$, *c*1 0), lit. [3] $[\alpha]_D$ +55 8° ($CHCl_3$; *c*1 0), IR ν_{max}^{KBr} cm^{-1}· 3430, 1742, 1710, 1695 (sh), 1630, 1460, 1365, 1305, 1265, 1250, 1225, 1160, 1095, 1080, 1060, 910, 855, 762, 705, FDMS *m/z*· 1009 $[M+Na]^+$, base peak; UV λ_{max}^{MeOH} nm 216, 221 and 278 (logε 4 43, 4 38 and 4.62)

^1H NMR ($CDCl_3$). δ0 93 (3H, *s*, H_3-19), 1.08 (3H, *s*, H_3-18), 1 21, 1 25, 1 34 (3H each, *d*, *J* = 6 2, 6.2, 5.4 Hz), 1.84 (3H, *s*, Ac), 2.12 (3H, *s*, H_3-21), 3.06 (1H, *m*, H-17α), 3.37, 3 42, 3.64 (3H each, OMe), 4 45 (1H, *dd*, *J* = 1 6, 10 Hz, anomeric), 4 65 (1H, *d*, *J* = 8.1 Hz, anomeric), 4 77 (1H, *d*, *J* = 8.3 Hz, anomeric), 4 82 (1H, *dd*, *J* = 1.6, about 10 Hz, anomeric overlapped), 4.84 (1H, *d*, *J* = about 10 Hz, H-12α overlapped), 5.31 (1H, *t*, *J* = 10 Hz, H-11β), 6.34, 7 73 (1H each, *J* = 16 Hz AB), 7.41, 7 55 (3H, 2H arom)

Condurangoglycoside E (**2**). White amorphous material, mp 129–133°, $[\alpha]_D$ +68 5° ($CHCl_3$; *c* 1 0) (Found. C, 61.53, H, 7.77 $C_{53}H_{76}O_{18}$ $2H_2O$ requires. C, 61.37, H, 7.77%), IR ν_{max}^{KBr} cm^{-1}. 3440, 1740, 1708, 1690, 1630, 1445, 1370, 1265, 1220, 1155, 1095, 1075, 1055, 905, 855, 762, 705, FDMS *m/z* 1023 $[M+Na]^+$, 142 (base peak), UV λ_{max}^{MeOH} nm. 216, 222 and 278 nm (logε 4 15, 4 13 and 4.22), ^1H NMR ($CDCl_3$) δ1 22 (3H, *s*, H_3-19), 1 30 (3H, *s*, H_3-18), 1 21, 1 24, 1 34 (3H each, *J* = 6.2, 6 2 and 5.4 Hz), 1.89 (3H, *s*, Ac), 2 15 (3H, *s*, H_3-21), 3.07 (1H, *m*, H-17α), 3 37, 3 43, 3 65 (3H each, *s*, OMe), 4 45 (1H, *dd*, *J* = 2, 10 Hz, anomeric), 4 65 (1H, *d*, *J* = 8 1 Hz, anomeric), 4.77 (1H, *d*, *J* = 8.7 Hz, anomeric), 4 84 (1H, *dd*, *J* = 2, 10 Hz, anomeric), 4 99 (1H, *d*, *J* = 10 Hz, H-12α), 5 37 (1H, *m*, H-6), 5.83 (1H, *t*, *J* = 10 Hz, H-11β), 6 45, 7 75 (1H each, *J* = 16 Hz AB), 7 41, 7 56 (3H, 2H, arom).

Condurangoglycoside C (**9**) White amorphous material, mp 140–143°, $[\alpha]_D$ +32° ($CHCl_3$, *c*1 0), lit. [3] $[\alpha]_D$ +16.1° ($CHCl_3$, *c* 0.9), IR ν_{max}^{KBr} cm^{-1}· 3420, 1740, 1710, 1630, 1450, 1365, 1305, 1270, 1250, 1160, 1090–1050, 910, 860, 765; FDMS *m/z* 1011 $[M+Na]^+$, base peak, UV λ_{max}^{MeOH} nm. 215, 220 and 278 (logε 4 43, 4 37 and 4 58); ^1H NMR ($CDCl_3$)· δ0 94 (3H, *s*, H_3-19), 1.31 (3H, *s*, H_3-18), 1 18 (3H, *d*, *J* = 6 7 Hz, H_3-21), 1 21, 1 25, 1 33 (3H each, *d*, *J* = 6 2, 6 2, 5 4 Hz), 1 83 (3H, *s*, Ac), 3.36, 3.42, 3.64 (3H each, OMe), 4 45 (1H, *dd*, *J* = 1 7, 10 Hz, anomeric), 4 77 (1H, *d*, *J* = 8 3 Hz, anomeric), 4 85 (2H, anomeric overlapped, H-12α), 5 30 (1H, *t*, *J* = 10 Hz, H-11β), 6 40, 7 68 (1H each, *J* = 16 Hz AB), 7 38, 7 52 (3H, 2H, arom)

Isolation of the condurangoglycosides **5**, **8**, **3** and **10**. Nine separations of 90 g of the Et_2O insoluble portion on silica gel (0 05–0 2 mm) with $CHCl_3$–MeOH (0.0, 5 0, 10 0, 20.0% MeOH) yielded 15 4 g of the mixture of **5**, **8**, **3** and **10**. Rechromatography of 10 g of this glycosidic mixture on silica gel (0.04–0 063 mm) with $CHCl_3$–MeOH (5.0, 7 5, 10 0% MeOH) resulted in four subfractions (3–6, 5 6 g), which the pure compounds were isolated from by means of twofold prep HPLC on RP silica gel with MeCN–H_2O, as mentioned before. The pure glycosides obtained thus are summarized with increasing polarity, according to their chromatographic behaviour on RP silica gel.

Condurangoglycoside E_3 (**5**). White amorphous material, mp 168–172°; $[\alpha]_D$ +68° ($CHCl_3$, *c*1 0) (Found C, 58 94, H, 8 12. $C_{66}H_{98}O_{26}$·$2H_2O$ requires C, 59 00, H, 7 65%); IR ν_{max}^{KBr} cm^{-1} 3430, 1745, 1710, 1695, 1632, 1450, 1365, 1265, 1250, 1225, 1160, 1100–1050, 900, 860, 765, 705, FDMS *m/z* 1289 $[M-H_2O+1]^+$, 1270 $[M-2H_2O]^+$ base peak; UV λ_{max}^{MeOH} nm 216, 221 and 278 (logε 4 15, 4 09 and 4.43); ^1H NMR ($CDCl_3$) δ1.14 (3H, *s*, H_3-19), 1.22 (3H, *s*, H_3-18), 1 12, 1 13, 1 20, 1 26 (3H each, *d*, *J* = 6 2, 6 2, 6 2 and 5 4 Hz), 1 82 (3H, *s*, Ac), 2 08 (3H, *s*, H_3-21), 2 99 (1H, *m*, H-17α), 3.30, 3 34, 3.35, 3 51 (3H each, OMe), 4 26 (1H, *d*, *J* = 7 8 Hz, anomeric), 4.39 (1H, *dd*, *J* = 2, 10 Hz, anomeric), 4 65 (1H, *d*, *J* = 8 1 Hz, anomeric), 4 66 (1H, *dd*, *J* = 1 8, about 10 Hz, anomeric), 4 75 (1H, *br d*, *J* = about 10 Hz, anomeric), 4 91 (1H, *d*, *J* = 10 Hz, H-12α), 5 29 (1H, *m*, H-6), 5 74 (1H, *t*, *J* = 10 Hz, H-11β), 6 38, 7 67 (1H each, *J* = 16 Hz AB), 7 34, 7.49 (3H, 2H, arom)

Condurangoglycoside A_0 (**8**). White amorphous material, mp 166–168°, lit. [5] 170–174°, $[\alpha]_D$ +51° ($CHCl_3$; *c*1 0), lit [5] $[\alpha]_D$ +43 9 (MeOH, *c* 0 62); IR ν_{max}^{KBr} cm^{-1} 3430, 1745, 1715, 1700 (sh), 1635, 1455, 1370, 1310, 1260, 1230, 1165, 1100–1060, 770, FDMS *m/z* 1171 $[M+Na]^+$, UV λ_{max}^{MeOH} nm. 216, 221 and 278 (logε 4.43, 4.37 and 4.68), ^1H NMR ($CDCl_3$) δ0.92 (3H, *s*, H_3-19), 1 08 (3H, *s*, H_3-18), 1.20, 1 26, 1 32 (3H each, *d*, *J* = 6 2, 6 2, 5 7 Hz), 1.83 (3H, *s*, Ac), 2.12 (3H, *s*, H_3-21), 3.05 (1H, *m*, H-17α), 3 36, 3.41, 3.57 (3H each, OMe), 4 36 (1H, 7 6 Hz, anomeric), 4 44 (1H, *dd*, anomeric, not resolved), 4 75 (1H, *d*, *J* = 7 8 Hz, anomeric), 4 81 (1H, *dd*, anomeric overlapped), 4 83 (1H, *d*, *J* = about 10 Hz, H-12α overlapped), 5 31 (1H, *t*, *J* = 10 Hz, H-11β), 6 43, 7 72 (1H each, *J* = 16 Hz AB), 7.40, 7 54 (3H, 2H arom.)

Condurangoglycoside E_0 (**3**) White amorphous material, mp 165–169°, $[\alpha]_D$ +69 0° ($CHCl_3$; *c*1 0) (Found C, 59 63; H, 8.22 $C_{59}H_{86}O_{23}$·$2H_2O$ requires C, 59 08, H, 7 50%), IR ν_{max}^{KBr} cm^{-1} 3440, 1750, 1715, 1700, 1645, 1455, 1385, 1370, 1270 (sh), 1255, 1230, 1165, 1105–1060, 910 (*br*), 865, 770, 710; FDMS *m/z*: 1185 $[M+Na]^+$, 1163 $[M+H]^+$, UV λ_{max}^{MeOH} nm 216, 221 and 278 (logε 4 27, 4 22 and 4 39), ^1H NMR ($CDCl_3$) δ1 24 (3H, *s*, H_3-19), 1.32 (3H, *s*, H_3-18), 1.20–1 34 (9H integrating), 1.90 (3H, *s*, Ac), 2.17) (3H, *s*, H_3-21), 3.37, 3.44, 3 59 (3H each, OMe), 4 2–4 8 (4H, anomeric), 5 00 (1H, *d*, *J* = 10 Hz, H-12α), 5 37 (1H, *m*, H-6), 5 84 (1H, *t*, *J* = 10 Hz, H-11β), 6.46, 7 77 (1H each, *J* = 16 Hz AB), 7 43, 7.58 (3H, 2H, aromatic).

Condurangoglycoside D_0 (**10**) White amorphous material mp 173–176°, lit [6] 183–88°, $[\alpha]_D$ −0 2 ($CHCl_3$; *c*1 0), lit [6] $[\alpha]_D$ +13 5° ($CHCl_3$, *c* 0.99), IR ν_{max}^{KBr} cm^{-1} 3400, 1740, 1710, 1630, 1447, 1365, 1305, 1250, 1225, 1155, 1090, 1055, 885, 865, 765, 705, FDMS *m/z* 1187 $[M+Na]^+$, base peak, UV λ_{max}^{MeOH} nm· 216, 221 and 278 (logε 4.28, 4 22 and 4 55), ^1H NMR ($CDCl_3$) δ0 87–1 32 (5 × Me, not resolved), 1 88 (3H, *s*, Ac), 3.37, 3 43, 3 58 (3H each, OMe), 6.43, 7 72 (1H each, *J* = 16 Hz, AB), 7 41, 7 55 (3H, 2H arom.).

Condurangogenin A (**11**) ^1H NMR ($CDCl_3$) δ0 97 (3H, *s*, H_3-19), 1 11 (3H, *s*, H_3-18), 1 87 (3H, *s*, Ac), 2 14 (3H, *s*, H_3-21), 3 08 (1H, *m*, H-17α), 3 56 (1H, *m*, H-3α), 4 86 (1H, *d*, *J* = 10 Hz, H-12α), 5 34 (1H, *t*, *J* = 10 Hz, H-11β, 6 45, 7 74 (1H each, *J* = 16 Hz AB), 7 43, 7 56 (3H, 2H, arom.)

Hydrolysis of condurango glycosides (i) Mild acidic hydrolysis To each 3 mg glycoside 1 ml MeOH and 2 ml 0 1 N HCl were added and the mixture kept for 30 min at 60° After evapn under

red pres, 3 ml 0 1 N HCl were added, and the mixture kept for 30 min at 60° again Neutralization with ion exchanger Amberlite IRA 410, filtration and evapn to dryness gave a residue to which 0 3 ml MeOH–H_2O (1 1) was added In the case of glycosides without a terminal glucose moiety, pachybiose and cymarose could be identified by TLC in the system EtOAc–MeOH (98 2) after spraying with thymol–H_2SO_4 reagent, when compared with authentic samples

(ii) Stronger acidic hydrolysis To each 3 mg glycoside 3 ml 0 1 N HCl were added, and the mixture refluxed for 30 min After neutralization with ion exchanger, filtration and evapn under red. pres the residue was dissolved in 0 3 ml MeOH–H_2O (1 1) In the case of glycosides without a terminal glucose moiety, 3-*O*-methyl-6-deoxyallose, oleandrose and cymarose could be identified under the same conditions as mentioned under (1)

Glycosides with a terminal glucose moiety were treated in the same way Glucose and 3-*O*-methyl-6-deoxyallose could be identified from the residue of stronger acidic hydrolysis by enzymatic cleavage with β-glucosidase and subsequent TLC in the system $CHCl_3$–MeOH–H_2O (75 24 1)

(iii) CG E (**2**) was hydrolysed following method (ii) to give condurangogenin E (**1**) The aqueous layer was shaken with *n*-hexane exhaustively and the combined organic layers were evapd to dryness

Condurangogenin E (**1**) White material from $CHCl_3$–MeOH, mp 107–109°, $[\alpha]_D$+ 105° ($CHCl_3$, *c* 0 75), IR ν_{max}^{KBr} cm^{-1} 3460, 1745, 1710, 1700, 1635, 1450, 1380, 1365, 1330, 1310, 1270 (sh), 1250, 1225, 1205, 1165, 1060 (sh), 1030, 965, 905, 860, 765, 705, FDMS *m/z* 552 $[M]^+$, base peak, UV λ_{max}^{MeOH} nm: 216, 222 and 280 ($\log\varepsilon$ 4 47, 4 42 and 4 62), ^{1}H NMR ($CDCl_3$) δ1 25 (3H, *s*, H_3-19), 1 31 (3H, *s*, H_3-18), 1 90 (3H, *s*, Ac), 2 16 (3H, *s*, H_3-21), 3.08 (1H, *m*, H-17α), 3 53 (1H, *m*, H-3α), 5 00 (1H, *d*, *J* = 10 Hz, H-12α), 5 38 (1H, *m*, H-6), 5.85 (1H, *t*, *J* = 10 Hz, H-11β), 6 45, 7 76 (1H each, *J* = 16 Hz AB), 7 42, 7 57 (3H, 2H, arom)

Drevogenin D (**12**) ^{1}H NMR ($CDCl_3$). δ0 91 (3H, *s*, H_3-19), 0 97 (3H, *s*, H_3-18), 1.02 (3H, *d*, *J* = 6.5 Hz, H_3-21), 2 82 (1H, *d*, *J* = 10 Hz, H-12α), 3 25 (1H, *m*, H-3α), 3 57 (1H, *dq*, *J* = 6 4 Hz, H-20), 3 65 (1H, *t*, *J* = 10 Hz, H-11β), 5.24 (1H, *m*, H-6)

Acetylation of Drevogenin D (**12**) Compound **12** (281 8 mg) was acetylated with pyridine–Ac_2O, according to ref [11] TLC analysis with $CHCl_3$–MeOH (95 5) showed two bluish spots with *p*-anisaldehyde–H_2SO_4 reagent and heating (ratio *ca* 4 1) CC of the raw acetates on silica gel (0 04–0 063 mm) with $CHCl_3$–MeOH (95 5) yielded 25 1 mg of **14** and 124 9 mg of **13**, chromatographically almost pure material, and a mixture of both compounds (171 4 mg)

Drevogenin D 3,12,20-*triacetate* (**13**) ^{1}H NMR ($CDCl_3$) δ1 03 (3H, *s*, H_3-19), 1.16 (3H, *s*, H_3-18), 1 15 (3H, overlapped, H_3-21), 3 81 (1H, *t*, *J* = 10 Hz, H-11β), 4.60 (1H, *m*, H-3α), 4 62 (1H, *d*, *J* = 10 Hz, H-12α), 4 85 (1H, *m*, H-20), 5 48 (1H, *m*, H-6), 2 01, 2 02, 2 18 (3H each, *s*, Ac)

Drevogenin D 3,11,12,20-*tetraacetate* (**14**) ^{1}H NMR ($CDCl_3$) δ1 07 (3H, *s*, H_3-19), 1 13 (3H, *s*, H_3-18), 1 14 (3H, overlapped, H_3-21), 4 55 (1H, *m*, H-3α), 4 78 (1H, *d*, *J* = 10 Hz, H-12α), 4 84 (1H, *m*, H-20), 5 31 (1H, *t*, *J* = 10 Hz, H-11β), 5 49 (1H, *m*, H-6), 1 96, 2 00, 2 01, 2 07 (3H each, *s*, Ac)

Acknowledgements—We thank Professor Dr T Reichstein, Universitat Basel, for authentic samples of drevogenin D, pachybiose and 3-*O*-methyl-6-deoxyallose and Professor Dr P Welzel, Ruhr Universitat Bochum, for an authentic sample of condurangogenin A We are grateful to Dr K Steinbach, Fachbereich Chemie, Universitat Marburg, for field desorption mass spectral measurements The technical assistance of Mrs E Keese and Mr M. Bode is gratefully acknowledged

REFERENCES

1 Tschesche, R, Welzel, P and Snatzke, G (1965) *Tetrahedron* **21**, 1777
2. Tschesche, R, Welzel, P and Fehlhaber, H W. (1965) *Tetrahedron* **21**, 1797
3. Tschesche, R, Kohl, H and Welzel, P (1967) *Tetrahedron* **23**, 1461
4 Tschesche, R and Kohl, H (1968) *Tetrahedron* **24**, 4359
5 Hayashi, K, Wada, K, Mitsuhashi, H, Bando, H, Takase, M, Terada, S, Koide, Y, Aiba, T, Narita, T and Mizuno, D (1980) *Chem Pharm Bull* **28**, 1954
6 Hayashi, K, Wada, K, Mitsuhashi, H, Bando, H, Takase, M, Terada, S, Koide, Y, Aiba, T and Narita, T (1981) *Chem Pharm Bull* **29**, 2725
7 Pailer, M and Ganzinger, D (1975) *Mh Chem* **106**, 37
8 Saner, A, Stockel, K and Reichstein, T (1972) *Helv Chim Acta* **55**, 1221
9 Miyagawa, S, Yamaura, K, Hayashi, K, Kaneko, K and Mitsuhashi, H (1986) *Phytochemistry* **25**, 2861
10 Wada, K, Hayashi, K, Mitsuhashi, H and Bando, H (1982) *Chem Pharm Bull* **30**, 3500
11 Sauer, H H, Weiss, E and Reichstein, T (1965) *Helv Chim Acta* **48**, 857