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NEW LIGNANS FROM THE HEARTWOOD OF *CLEISTANTHUS COLLINUS*

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Abstract—The isolation and characterisation of five new compounds and nine known compounds from the heartwood of *Cleistanthus collinus* are reported. The new compounds are wodeshiol **16**, 3,4-dihydrotaiwanin C **20**, and three new glycosides **24**, **25** and **26** of diphyllin and taiwanin E. The ^1H and ^{13}C NMR spectra of these compounds are also reported.

Cleistanthus collinus Roxb. is well known for its extremely poisonous character.[1] Recent investigations have resulted in the isolation of the toxic 1-arylnaphthalene lactones, diphyllin (**1**)[2] and collinusin (**2**),[2,3] and three glycosides of diphyllin, herein referred to as cleistanthins A (**3**),[2] B(**4**)[4] and C(**5**).[5] These results prompted our examination of the heartwood constituents of *C. collinus*.

Fourteen pure crystalline compounds were isolated, nine of which were readily identified as diphyllin (**1**),[2] cleistanthin A (**3**),[2] cleistanthin C (**5**),[5] paulownin (**6**),[6] sesamin (**7**),[7] 4-hydroxysesamin (**8**),[8] dihydrocubebin (**9**),[9,10] taiwanin C (**10**)[11] and taiwanin E (**11**)[11,12] on the basis of their spectral data and by comparison with authentic samples.

The identification of dihydrocubebin (**9**), $C_{20}H_{22}O_6$, m.p. 112°, $[\alpha]_D$ − 42°, was further confirmed by its conversion into hinokinin (**12**)[9] by oxidation with silver carbonate on silica gel. Furthermore, methylation of dihydrocubebin with methyl iodide and silver oxide in dimethylformamide gave the methylenedioxy analogue (**13**) of phyllanthin.[13] Acetylation of dihydrocubebin gave a diacetate (**14**), while refluxing with methanolic HCl afforded the dibenzyltetrahydrofuran (**15**). The ^1H and ^{13}C NMR spectra of dihydrocubebin and its derivatives (Tables 1 and 2) are in full accord with the proposed structures.

Wodeshiol (**16**),[14] $C_{20}H_{18}O_8$, m.p. 153–154°, $[\alpha]_D$ − 12°, contains two hydroxyl groups and afforded a diacetate (**17**), m.p. 169–170°. The ^1H NMR spectrum (Table 3) indicated that wodeshiol contained a 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane skeleton,[8,15] but also showed that it had a highly symmetrical structure. Thus, in addition to the signals due to the aromatic protons the ^1H NMR spectrum contains only four singlets, due to the methylenedioxy protons, the 2/6 protons, the 4/8 protons, and the hydroxyl protons. However there are no high field signals such as those characteristic of H-5 in paulownin (**6**) or H-1 and H-5 in sesamin (**7**). In the ^1H NMR spectrum of the diacetate (**17**) the 4/8 protons appear as a pair of doublets at τ5.50 and 5.72 (J = 10 Hz). The spectrum of the diacetate also confirmed that there were no CHOH or CH_2OH groups in wodeshiol since none of the signals moved downfield by more than 0.3 ppm upon

Table 1. ^1H NMR spectra of dihydrocubebin and its derivatives*

Proton	Dihydrocubebin (9) ($CDCl_3$)	Dimethylether (13) ($CDCl_3$)	Diacetate (14) ($CDCl_3$)	Lactone (12) ($CDCl_3$)	Tetrahydrofuran (15) ($CDCl_3$)
1, 4	7.36 m	7.43 m	7.44 m	7.50 m	7.52 m
2, 3	8.18 m	7.98 m	7.94 m	7.12 m	7.91 m
α, α′	6.27 dd (2, 11) 6.57 dd (4, 11)	6.76 d	6.01 m	5.90 dd 6.20 dd	6.16 dd (6, 9) 6.57 dd (6, 8)
OCH_2O	4.18 s	4.15 s	4.18 s	4.13 s	4.19 s
arom.	3.3-3.6 m	3.3-3.6 m	3.3-3.6 m	3.3-3.6 m	3.3-3.6 m
OH	6.12 br. s	-	-	-	-
OAc	-	-	8.01 s	-	-
OMe	-	6.77 s	-	-	-

* Values in τ, coupling constants (Hz) in brackets. All assignments supported by appropriate spin decoupling experiments and correct integration.

Table 2. ^{13}C NMR spectra of dihydrocubebin and its derivatives*

Carbon	Dihydrocubebin (9)	Dimethyl ether (13)	Diacetate (14)	Lactone (12)	Tetrahydrofuran (15)
1 } 4 }	35.74	34.96	34.83	34.80 38.33	39.17
2 } 3 }	44.08	41.08	39.93	41.24 46.42	46.48
α } α¹ }	59.85	72.71	64.18	71.06 178.17	73.16
1'1" {	134.29	135.22	133.21	131.18 131.45	133.95
3'3" { 4'4" {	145.47 147.34	145.91 147.84	145.75 147.51	146.17 146.29 147.69	145.68 147.46
2'2" { 5'5" {	107.88 109.19	108.20 109.69	107.99 108.97	108.19 108.68 109.30	107.95 108.85
6'6" {	121.69	122.21	121.66	121.39 122.07	121.33
OCH_2O	100.59	100.96	100.73	100.88	100.69
OMe	-	58.83	-	-	-
OAc	-	-	170.80 20.90	-	-

* Measurements are given as p.p.m. downfield from TMS as internal standard at zero. All Assignments are supported by off-resonance decoupling experiments.

Spectra run in $CDCl_3$ solution.

acetylation. Since the 4/8 protons all come below 6τ it can be deduced that both aryl groups are equatorial.[16,17]

The ^{13}C NMR spectrum of wodeshiol (Table 4) contains only ten signals which can be readily assigned by comparison with the spectra of other 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octanes.[8,15] Thus the signal at 87.75 ppm due to C-1 and C-5 is seen as a singlet in the off-resonance spectrum, confirming that this is a quarternary centre. Once again there are no high field signals corresponding to those of C-5 in paulownin (**6**) or C-1 and C-5 in sesamin (**7**). The signal at 75.95 ppm is assigned to C-4 and C-8 since it becomes a triplet in the off-resonance spectrum, while the signal at 87.25 ppm due to C-2 and C-6 is a doublet, confirming that there are no additional substituents attached to these positions. The two hydroxyl groups are therefore attached to C-1 and C-5 and wodeshiol is therefore 1,5-dihydroxysesamin (**16**), a uniquely dihydroxylated lignan.

The mass spectrum of wodeshiol contains a number of fragment ions, notably *m/e* 236(M–ArCHO), *m/e* 177(ArCH=C(OH)CH_2) and *m/e* 162 (ArCH=C=O) which are characteristic of the spectra of 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octanes[8,15] and support the proposed structure (**16**) (see Experimental section).

Periodate oxidation of wodeshiol was complete in 4 hr and gave a crystalline compound, $C_{20}H_{18}O_9$, m.p. 210°, $[\alpha]_D$ −203°, which unexpectedly showed no carbonyl band in its infrared spectrum but gave a broad hydroxyl peak at 3300 cm $^{-1}$. The ^1H NMR spectrum confirmed that this product was the cyclic hemiketal (**19**), and the observation of long range coupling between the axial protons at C-2 and C-6 and those at C-4 and C-8 is consistent with the conformation shown. The molecular ion (*m/e* 384) observed in the mass spectrum in fact corresponds to the diketone (i.e. M–H_2O) rather than to the hemiketal.

Taiwanin C (**10**), $C_{20}H_{12}O_6$, m.p. 271°, was isolated along with a second compound which could only be separated from it by careful chromatography. This compound, $C_{20}H_{14}O_6$, m.p. 199–200°, gave an NMR spectrum (Table 5), which was very similar to that of collinusin (**2**),[18] and it is therefore assigned structure (**20**), corresponding to the 3,4-dihydro derivative of taiwanin C. Both taiwanin C and its 3,4-dihydro-derivative gave intense molecular ions as the main feature of their mass spectra (see Experimental section).

Cleistanthin A (**3**), $C_{28}H_{28}O_{11}$, m.p. 135–136°, $[\alpha]_D$ −63°, was also accompanied by second minor component which was separated from it by treatment with acetic anhydride and pyridine which afforded cleistanthin A acetate and the unacetylated minor component. The latter compound (cleistanthin D) (**24**), $C_{29}H_{30}O_{11}$, m.p. 199–200°, gave a prominent peak at 1755 cm^{-1} in its IR spectrum and gave diphyllin on acid hydrolysis. Its molecular weight corresponded to that of the monomethyl ether of cleistanthin A. However direct comparison with a sample of cleistanthin A methyl ether (**21**)[2] showed that the two compounds were clearly different (R_f, m.p. and NMR; see Tables 6 and 7). Furthermore, examination of the benzene induced solvent shifts of the methoxyl groups in the two compounds showed that only four of the five methoxyl groups were in similar environments. Although

Table 3. ^{1}H NMR spectra of paulownin, wodeshiol and derivatives*

Proton	Paulownin (6) ($CDCl_3$)	Paulownin acetate (18) ($CDCl_3$)	Wodeshiol (16) ($CDCl_3$)	Wodeshiol acetate (17) ($CDCl_3$)	Periodate product (19) ($CDCl_3$-DMSO)
1	-	-	-	-	-
5	7.02m	6.76m	-	-	-
2	5.26s	5.01s	5.04s	4.89s	5.60br.s
6	5.23d(6)	5.32d(5)			
4a	6.24dd(6,9)	6.28dd(5,9)	5.94s	5.72d(10)	6.69dd(11,1.5)
8a	6.17d(10)	5.83d(10)			
4e	5.56dd(8,9)	5.63dd(7,9)		5.60d(10)	6.25d(11)
8e	6.01d(10)	5.61d(10)			
OCH_2O	4.10s,4.12s	4.10s,4.12s	4.07s	4.11s	4.14s
Arom.	3.1-3.4m	3.1-3.4m	3.1-3.3m	3.1-3.4m	2.9-3.4m
OH	8.15s	-	7.55s	-	3.58br.s
OAc	-	8.29s	-	8.17s	-

* Values in τ, coupling constants (Hz) in brackets. All assignments supported by appropriate spin decoupling experiments and correct integration.

Table 4. ^{13}C spectra of paulownin, wodeshiol and derivatives*

Carbon	Paulownin (6)	Paulownin acetate (18)	Wodeshiol (16)	Wodeshiol acetate (17)	Periodate product (19)
1	91.74	97.08	87.75	92.31	94.90
5	60.58	58.95			
4	71.58	69.85	75.95	74.26	68.58
8	74.98	75.08			
2	87.48	86.75	87.25	86.83	82.30
6	85.88	85.67			
1'1"	129.38	130.11	130.95	129.97	131.42
	134.79	133.93			
		147.22			
3'3"4'4"	147.33	147.39	147.33	147.46	147.26
	148.21	147.91	147.11	147.83	147.33
		106.67			
2'2"5'5"	106.91	107.83	107.65	108.07	107.85
	107.49	108.07	108.30	109.22	108.56
	108.23	108.73			
	108.56				
6'6"	119.79	119.68	120.62	122.96	121.49
	120.14	122.18			
OCH_2O	101.12	100.98	100.88	101.20	100.94
	101.24	101.02			
OAc	-	169.20	-	168.97	-
		20.91		20.96	

* Measurements are given as p.p.m. downfield from TMS as internal standard at zero. All assignments are supported by off-resonance decoupling experiments. Solvents as in Table 3.

Table 5. ^{1}H NMR spectra of taiwanin C,3,4,-dihydro-taiwanin C and collinusin*

Proton	Taiwanin C (10) (DMSO)	3,4-dihydro taiwanin C (20) (DMSO)	Collinusin (2) ($CDCl_3$) [18]
3	-	7.14m	5.18-7.33m
4	2.19brs		
5	2.69s	2.9-3.65m	3.20br(4H)
8	3.14s		
2'	3.10d(8)		3.43s(H-8)
5'	3.27d(2)		
6'	3.35dd(2,8)		
CH_2	4.71d(2)[6]	5.31t(9),5.97t(9)	5.18-7.33m
OMe	-	-	6.36s,6.10s
OCH_2O	3.98s,4.02s	3.88s,3.94 AB	4.02s

* Values in τ, coupling constants (Hz) in brackets. All assignments supported by appropriate spin decoupling experiments and correct

Table 6. ^{1}H NMR spectra of diphyllin and cleistanthins A and D*

Proton	Diphyllin (1) ($CDCl_3$)	Cleistanthin A (3) ($CDCl_3$)	Cleistanthin A acetate ($CDCl_3$)	Cleistanthin A methylether (21) ($CDCl_3$)	Cleistanthin D (24) ($CDCl_3$)
5	2.54s	2.10s	2.40s		
8	2.88s	2.96s	2.96s		
2'	3.14s	3.20s	3.20s	2.16s and 2.9-3.3m	2.10s and 2.9-3.3m
5'	3.03dd(7,1)	3.06d(8)	3.06d(8)		
6'	3.18dd(7,2)	3.24dd(8,2)	3.24dd(2,8)		
CH_2	4.62s	4.57s	4.56br.s	4.58 AB	4.52br.s
OMe	5.91s,6.17s	5.97s,6.21s,6.32s, and 6.52s	5.91s,6.20s,6.38s and 6.48s	5.97s,6.22s,6.25s,6.37s and 6.56s	5.96s,6.23s,6.24s,6.47s and 6.51s
OCH_2O	3.92 AB	3.95 AB	3.95 AB	3.98 AB	3.98 AB
OH	-	8.2br	-	-	-
Sugar Protons	-	5.01d(5)and5.8-6.8m	4.86d(6),4.65d(6) and 5.8-6.8m	5.25d(7)and 6-7m	5.24d(7)and6-7m
OAc	-	-	-	-	-

* Values in τ, coupling constants (Hz) in brackets. All assignments supported by appropriate spin decoupling experiments and correct integration

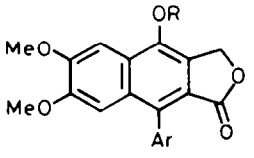
Table 7. ^{13}C spectra of diphyllin and cleistanthins A and D*

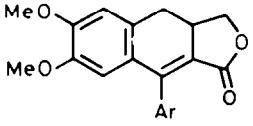
Carbon	Diphyllin (1)	Cleistanthin A (3)	Cleistanthin A acetate	Cleistanthin A methyl ether	Cleistanthin D (24)
1	118.76	119.16	?	119.23	119.4
2	121.67	126.94	126.00	127.04	123.64
3	123.51	128.45	126.15	128.37	128.46
4	128.99	129.17	128.41	130.78	127.10
9	129.71	130.68	130.68	130.99	130.73
10	129.66	135.96	135.60	136.34	136.06
6,7	149.84	150.26	150.40	150.19	150.17
	150.59	151.89	151.87	151.95	151.87
5,8	100.99	103.82	100.85	105.13	104.87
	111.04	110.79	110.74	110.70	110.71
1'	145.04	144.24	144.09	144.36	144.56
3'	146.72	147.51(x 2)	147.54(x 2)	147.51(x 2)	147.48(x 2)
4'	146.97				
2,5'	105.78	106.13	106.13	106.27	106.12
	107.73	108.17	108.20	108.13	108.08
6'	123.75	123.67	123.61	123.60	123.52
CH_2	66.58	67.39	66.98	67.33	67.60
C=O	169.65	169.92	169.70	169.73	169.83
sugar carbons		101.17	100.61	100.70	100.96
		71.48	71.51	79.39	74.40
		78.42	78.02	83.48	80.58
		82.57	81.26	85.49	82.45
		61.91	62.81	63.45	62.29
OCH_2O	100.99	101.25	101.25	101.25	101.22
OMe	55.60	55.81	55.83	55.80	55.74
	55.20	56.22	56.24	56.10	56.12
		58.02	58.54	58.69	57.41
		60.13	59.92	60.68	57.80
				61.24	61.32
OAc			21.15		
			?		

* Spectra run in $CDCl_3$ solution.

Table 8. Mass spectra of cleistanthin A methyl ether and cleistanthin D

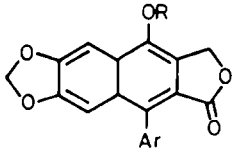
m/e	cleistanthin A methyl ether (21)	cleistanthin D (24)
554 (M^+)	1	8
522	1	-
382	6	2
381	32	20
380	100	81
379	4	3
351	3	2
335	3	-
322	2	2
321	7	5
307	3	-
294	3	3
293	14	12
249	2	2
221	-	2
219	3	-
187	4	-
176	3	7
175	25	69
174	15	9
163	4	2
151	2	-
150	3	2
145	-	2
144	6	8
143	76	100
117	4	4
116	26	19
115	22	27
113	3	3
112	-	2
111	12	13
102	5	5
101	76	90

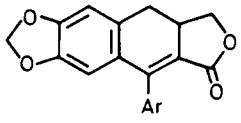




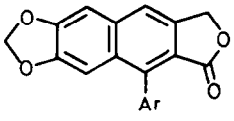
2.

1 R = H
3. R = 3,4-di-O-methyl-D-xylose
4. R = D-glucose
5. R = β-2,3-di-O-methyl-D-xylopyranosyl-(1→4)-β-D-glucopyranose
21 R = 2,3,4-tri-O-methyl-D-xylose
24 R = 2,3,5-tri-O-methyl-D-xylose
26. R = 2,3,5-tri-O-methyl-D-xylofuranosyl-2,3-di-O-methyl-D-xylopyranosyl-(1→4)-D-glucopyranose



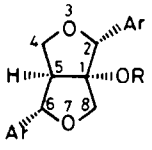


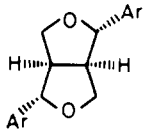
11. R=H 20.
25. R=3,4-di-O-methyl-D-xylose

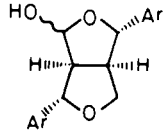


10.

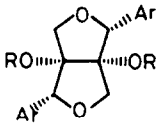
(Ar = 3,4-methylenedioxyphenyl)

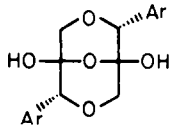


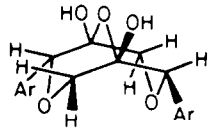




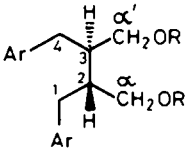
6. R = H
18 R = Ac 7. 8.

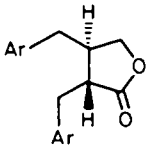


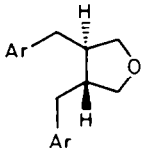




16 R = H
17 R = Ac 19.

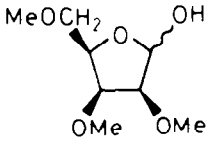




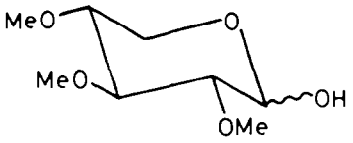


9 R = H
13 R = Me
14 R = Ac 12. 15.

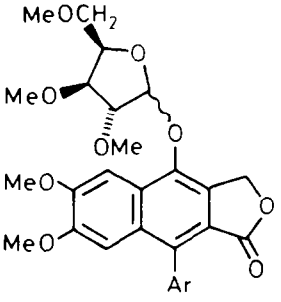
(Ar = 3,4-methylenedioxyphenyl)



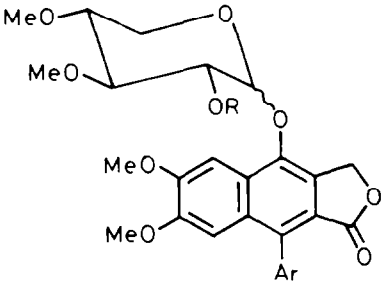
(22)



(23)



(24)



(3) R = H
(21) R = Me

the mass spectra contained the same fragment ions there were noticeable differences in their relative abundances, when run under the same conditions (Table 8), suggesting that cleistanthin D was simply an isomer of cleistanthin A methyl ether.

Indeed, hydrolysis of cleistanthin D with 7% methanolic hydrochloric acid was complete in 6 hr and afforded 2,3,5-tri-O-methylxylose (**22**), whereas hydrolysis of cleistanthin A methyl ether under the same conditions required approx. 12 hr and afforded 2,3,4-tri-O-methylxylose (**23**). The two xylose derivatives were identified by comparing their physical properties with values reported in the literature[19] (see Experimental section). Furthermore the relative retention times of the methyl glycosides of the two sugars on a 15% diethylene glycol succinate column were 1.61:1.00 which can be compared with the ratio of 1.60:1.00 reported for the methyl glycosides of β-2,3,5-tri-O-methylxylose and β-2,3,4-tri-O-methylxylose on a 10% carbowax 6000 column.[20]

Cleistanthin E, $C_{42}H_{52}O_{20}$, m.p. 177–80°, $[\alpha]_D - 40°$, formed a triacetate, m.p. 192°, $[\alpha]_D - 42$, and a tri-O-methyl ether, m.p. 140–142°, $[\alpha]_D - 36°$. Hydrolysis with 7% methanolic hydrochloric acid afforded diphyllin, D-glycose, 2,3-di-O-methylxylose and 2,3,5-tri-O-methylxylose, which were identified by co-chromatography with authentic samples. To establish the sequence of the three monosaccharides the cleistanthin E was first permethylated with methyl iodide and silver oxide in dimethyl formamide and then hydrolysed with 7% methanolic hydrochloric acid which gave 2,3,6 - tri - O - methyl - D - glucose, 2,3-di-O-methylxylose and 2,3,5-tri-O-methylmethylxylose. From this result it can be deduced that the 2,3,5 - tri - O - methylxylose (**22**) is the terminal sugar in the sequence but the order of the other two monosaccharides is undecided. In order to settle this point cleistanthin E was treated with 0.05 N H_2SO_4 in aqueous methanol at 60° for 12 hr which yielded only 2,3,5 - tri - O - methylxylose and 2,3 - di - O - methylxylose but no glucose, thus confirming that 2,3 - di - O - methylxylose is the middle sugar in the trisaccaride unit and that D-glucose is the sugar directly attached to the diphyllin nucleus. Cleistanthin E is therefore diphyllin 2,3,5 - tri - O - methyl - D - xylofuranosyl - 2,3 - di - O - methyl - D - xylopyranosyl(1→4) - D - glucopyranoside (**26**). Further confirmation of this structure was not possible due to the small amount of material available.

The third new glycoside, $C_{27}H_{24}O_{11}$, m.p. 210–212°, $[\alpha]_D + 11°$, formed a monoacetate, m.p. 200–220°, and a monomethyl ether, m.p. 248–250°. Hydrolysis of the glycoside yielded taiwanin E (**11**) and 3,4-di-O-methylxylose, while hydrolysis of the monomethyl ether of the glycoside again gave taiwanin E along with 2,3,4 - tri- O - methylxylose, each of which were identified by comparison with authentic samples. The 2,3,4 - tri - O - methylxylose was further converted into its lactone which was identical (m.m.p. and $[\alpha]_D$) with an authentic sample of 2,3,4-tri-O-methylxylonolactone. The structure of the glycoside is therefore taiwanin E 3,4-di-O-methyl-D-xylopyranoside (**25**).

The chemistry and distribution of lignans have been recently renewed.[21] It is clear[22] that the co-occurrence of phenyltetralin/naphthalene lignans with lignans of the furofuran type is rare, having up to now been reported in closely related species of *Picea* and in *Macropiper excelsum*. It is particularly interesting that *Gmelina asiatica* (Verbenaceae)[23] which produces hydroxylated furofurans also yields cycloolivil a hydroxylated phenyltetralin. *Cleistanus collinus* is the first member of the Euphorbaceaea to produce both classes of lignans, a phenomenon of some biosynthetic significance considering the possible common biosynthetic origin of both classes of lignan.[24] In the present case the co-occurrence is particularly noteworthy due to the high oxidation level of many of the extractives.

Many of the lignans reported here are more hydrophilic than the usual lignans either due to extra hydroxylation or to glycoside formation. Heavily oxygenated lignans of both classes such as podophylotoxin[25] or phrymarolin I[26] have important physiological activity as do certain lignan glycosides such as pinoresinol diglucoside[27] and liriodendrin[28]. Clearly the hydrophilic-

lipophilic balance is important for physiological activity and it will be of interest to see whether the poisonous properties of *C. collinus* are associated with any of the compounds reported.

EXPERIMENTAL

Mass spectra were recorded on an AE1 MS9 double focussing mass spectrometer and NMR spectra of Varian HA 100 and XL 100 instruments. IR Spectra were run on a Perkin–Elmer 237 spectrophotometer and UV Spectra on a Beckmann DB-G instrument. Silica gel C was used for TLC and the superscripts a, b, c, d and e indicate the solvent systems used (a = benzene–ethyl acetate 9:1, b = benzene–ethyl acetate 19:1, b = benzene–ethyl acetate 19:1, c = benzene–ethyl acetate 4:1, d = benzene–ethyl acetate 3:2, and e = chloroform–methanol 9:1).

Extraction of heartwood of Cleistanthus collinus Roxb.

The heartwood of *Cleistanthus collinus Roxb.* was collected from the Punyagiri hills of Andhra Pradesh 50 km from Visakhapatnam. The heartwood powder (15 kg) was extracted with methanol in a large soxhlet apparatus. The alcohol was evaporated under reduced pressure and the gummy mass obtained (1 kg) was absorbed on spent powder and successively extracted with hexane and chloroform.

The light green hexane extract (3 l) was concentrated to 500 ml. A white solid (3 g) separated, was filtered, and identified as β-sitosterol by comparison with an authentic sample. The mother liquor was concentrated under reduced pressure to give a reddish brown gum (10 g) which showed a number of spots on TLC and was not pursued further.

The pale green chloroform extract (5 l) showed eight clear spots on TLC. The chloroform was completely removed under reduced pressure and the pale green gum (50 g) extracted with benzene. The benzene soluble portion, obtained as a brown gum after removal of the solvent, showed eight clear spots on TLC. The gum (30 g) was absorbed on silica gel (200 g, finer than 200 mesh) and the dry powder placed on a column of silica gel (500 g) set in hexane. The column was eluted with hexane, benzene, benzene–ethyl acetate (9:1) and finally benzene–ethyl acetate (4:1). The elution was monitored by TLC while collecting litre fractions. The fractions and their composition as monitored by TLC are given in Table 9.

Fractions 1–10 yielded an oil (5 g) which was not pursued further. Fractions 11–20 were combined and the residue after evaporation recrystallised from chloroform, when compound A (600 mg) was obtained as colourless needles, m.p. 235°. Fractions 21–25 were combined and the residue after evaporation recrystallised from methanol–hexane when compound B (200 mg) came out as shining colourless crystals, m.p. 118°. Fractions 26–40 were mixed and the residue after evaporation recrystallised from benzene when compound C (3.0 g) came out as shining colourless needles, m.p. 102°. Fractions 41–45 were mixed and the residue after evaporation recrystallised from alcohol to give more of compound C (200 mg). The residue from these fractions on further concentration and recrystallisation from methanol yielded compound D (100 mg) as shining colourless plates m.p. 162°. Fractions 46–55 were mixed and the residue after evaporation recrystallised from benzene when compound E (3.0 g) was obtained as colourless needles, m.p. 153–154°. Fractions 56–60 were mixed and the residue after evaporation recrystallised from alcohol when more of compound E (200 mg) came out as colourless needles, m.p. 153–154°. The residue from these fractions on further concentration yielded only a mixture of compounds E, F and G. As it was only obtained in small quantity further separation of these three compounds was not attempted. Fractions 61–70 were mixed and the residue after evaporation recrystallised from alcohol when compound G (2 g) came out as colourless cubes, m.p. 112°. Fractions 71–80 were mixed and the residue after evaporation recrystallised from alcohol when compound H (500 mg) came out as colourless flakes, m.p. 291°. Fractions 81–90 were mixed and the residue after evaporation recrystallised from a mixture of benzene, ether and hexane, when a mixture (3 g) of two compounds I and J, was obtained as colourless buttons, m.p. 138–140°.

Fractions 91–95 were mixed and the residue after evaporation recrystallised from a mixture of methanol and chloroform, when compound K (300 mg) came out as colourless needles, m.p. 210–212°.

Fractions 96–100 were mixed and the residue after evaporation recrystallised from alcohol when compound L (200 mg) came out as colourless plates, m.p. 304°.

Fractions 101–105 were mixed and the residue after evaporation recrystallised from a mixture of chloroform and methanol when compound M (300 mg) came out as colourless buttons, m.p. 178°.

Fractions 106–110 were mixed and the residue after evaporation recrystallised from a mixture of chloroform and methanol giving compound N (300 mg) as colourless plates m.p. 220–221°.

Table 9. Analysis of *C. collinus* extract

Eluent	Fraction	Compounds present	Yield
Hexane	1 - 10	oil	5g
Benzene	11 - 20	A	600mg
	21 - 25	B	200mg
	26 - 40	C	3g
	41 - 45	C,D	300mg
	46 - 55	E	3g
	56 - 60	E,F,G	minor
Benzene-ethyl acetate 9:1	61 - 70	G	2g
Benzene-ethyl acetate 4:1	71 - 80	H	500mg
	81 - 90	I,J	3g
Benzene-ethyl acetate 7:3	91 - 95	K	300mg
	96 - 100	L	200mg
	101 - 105	M	300mg
	106 - 110	N	300mg

Examination of compound A

Compound A crystallised from benzene–chloroform as colourless flakes, m.p. 235°. It showed a single spot on TLC having R_f 0.68.[a] The NMR spectrum of this compound suggested that it was a mixture of two similar compounds (A1 and A2) and TLC showed two spots having R_f 0.54[b] and 0.50[b]. The two compounds were separated on a long column (length 100 cm, diameter 5 cm) of silica gel, using hexane–benzene (1:4) as eluent.

Identification of compound A1: Taiwanin C (**10**)

This substance crystallised from benzene as colourless plates, m.p. 271°, R_f 0.54[b]. (Found: C, 68.92; H, 3.51. $C_{20}H_{12}O_6$ requires C, 68.7; H, 3.47%). (Found: M^+ 348.0634. $C_{20}H_{12}O_6$ requires 348.0634). ν_{max} (Nujol): 1755 (γ-lactone), 1600 (arom), and 925 (OCH_2O)cm^{-1}. λ_{max} ($CHCl_3$): 352, 315 and 260 nm (log ε 4.13, 4.02 and 4.74). *m/e* 348(100), 261(15), 159(12). Labat test positive for OCH_2O, red colouration with conc. H_2SO_4. Accurate mass measurement: *m/e* 261.0552 ($C_{17}H_9O_3$). The IR, UV and ^1H NMR spectra were identical with those of taiwanin C (m.m.p. 276°).[11]

Identification of compound A2: 3,4-dihydrotaiwanin C (**20**)

This substance crystallised from benzene as colourless flakes, m.p. 199–200°, R_f 0.50[b] (Found: C, 68.50; H, 4.12. $C_{20}H_{14}O_6$ requires C, 68.57, H, 4.03%). (Found: M^+ 350.0790. $C_{20}H_{14}O_6$ requires 350.0790. ν_{max} (Nujol): 1730 (γ-lactone), 1630 (arom.), 1585, 925 (OCH_2O) and 850 cm^{-1}. *m/e* 350(100), 305(13), 275(13), 262(9), 176(13). Accurate mass measurements: *m/e* 305.0812 ($C_{19}H_{13}O_4$), 275.0708, ($C_{18}H_{11}O_3$), 262.0630 ($C_{17}H_{10}O_3$), 176.0626 ($C_{14}H_8$). When the substance is treated with conc. H_2SO_4 the solution turns first blue, then pink, and finally reddish brown.

Dehydrogenation of 3,4-dihydrotaiwanin C (**20**)

An intimate mixture of 3,4-dihydrotaiwanin C (100 mg) and palladium–charcoal (10%, 200 mg) was heated at 190–200° for 30 min, cooled and extracted with chloroform to yield taiwanin C (60 mg), m.m.p. 268–270°, R_f 0.54[b] (Found: C, 68.85; H, 3.54. $C_{20}H_{12}O_6$ requires C, 68.7; H, 3.47%).

Identification of compound B: Sesamin (**7**)

The substance was obtained as a light yellow oil which crystallised from hexane–methanol (1:1) as colourless crystals, m.p. 118° (lit[7] 123°), $[\alpha]_D$ + 71°, R_f 0.63[b], ν_{max}(Nujol) 1600 (arom.), 930 (OCH_2O)cm^{-1}.

Identification of compound C: Paulownin (**6**)

The substance crystallised from benzene as colourless needles, m.p. 102°, $[\alpha]_D$ + 28°, R_f 0.56[c]. Labat test positive for OCH_2O. It was found to be identical with paulownin (m.m.p. and IR). (Lit[6] m.p. 104–105°, $[\alpha]_D$ + 29°). With Ac_2O/pyridine an acetate (**18**), m.p. 145–146° (lit[6] 144–145°) was obtained.

Identification of compound D: 4-Hydroxysesamin (**8**)

The substance crystallised from benzene as colourless needles, m.p. 162°, $[\alpha]_D$ + 58°, R_f 0.40[c]. Labat test positive for OCH_2O. The compound was found to be identical with 4-hydroxysesamin (m.m.p. and IR) (lit[8] m.p. 165°, $[\alpha]_D$ + 58.4°). With Ac_2O/pyridine an acetate, m.p. 145° was obtained.

Identification of compound E: Wodeshiol (**16**)

The substance crystallised from benzene and also from methanol as colourless needles, m.p. 153–154°, $[\alpha]_D$ − 12°, R_f 0.39[c] (Found: C, 62.04, H, 4.86. $C_{20}H_{18}O_8$ requires C, 62.18; H, 4.70%). (Found M^+ 386.1002. $C_{20}H_{18}O_8$ requires 386.1002). ν_{max} (Nujol): 3480 (OH), 1615 (arom.) 930 (OCH_2O)cm^{-1}. *m/e* 386 (45), 236(3), 218(12), 179(9), 177(6), 163(7), 162(41), 152(12), 151(100), 150(24), 149(42), 135(43).

Reactions of Wodeshiol (**16**)

Acetylation of wodeshiol. Wodeshiol (100 mg) was heated with acetic anhydride (2.0 ml) and pyridine (10 ml) on a water bath for 3 h. and the product worked up in the usual way. Wodeshiol acetate (**17**) crystallised from methanol as shining needles (50 mg), m.p. 169–170°, R_f 0.68[c]. (Found: C, 61.25; H, 4.87. $C_{24}H_{22}O_{10}$ requires C, 61.28; H, 4.71%). (Found: M^+ 470.1212. $C_{24}H_{22}O_{10}$ requires 470.1215). ν_{max} (Nujol): 1740, 1620, 1600 and 930 cm^{-1}. *m/e* 470(10), 260(26), 219(12), 218(71), 217(49), 162(9), 151(44), 150(9), 149(16), 135(14). Accurate mass measurements: *m/e* 260.0690 ($C_{14}H_{12}O_5$), 218.0573($C_{12}H_{10}O_4$), 187.0396($C_{11}H_7O_3$), 177.0558($C_{10}H_9O_3$), 162.0319($C_9H_6O_3$).

Periodate oxidation of wodeshiol. Wodeshiol (100 mg) was dissolved in dioxane (10 ml) and an aqueous solution (5 ml) of potassium periodate (100 mg) added. The reaction was complete in 4 hr at room temperature (change in R_f from 0.39 to 0.12). The reaction mixture was poured into water (200 ml) and thoroughly extracted with ether. The ether layer was washed with dil aq $NaHCO_3$ and water, dried over $MgSO_4$ and evaporated. The product (**19**) was obtained as a white crystalline solid which crystallised from chloroform as needles (70 mg), m.p. 210°, $[\alpha]_D$ − 203°. (Found: C, 59.68; H, 4.60. $C_{20}H_{18}O_9$ requires C, 59.7; H, 4.51%.) ν_{max} (Nujol): 3410, 3300(OH), 1610(arom.) and 930(OCH_2O)dm^{-1}. *m/e* 384(11), 234(49), 164(19), 151(14), 150(13), 149(58), 148(51), 147(20), 136(10), 135(100), 134(11), 122(37), 121(12). Accurate mass measurements: *m/e* 384.0845 ($C_{20}H_{16}O_8$), 328.0950 ($C_{18}H_{16}O_6$), 234.0528($C_{12}H_{10}O_5$).

Oxidation of wodeshiol. Wodeshiol (100 mg) was dissolved in glacial acetic acid (2 ml), chromium trioxide (100 mg) added, and the mixture left overnight at room temperature. The excess chronium trioxide was destroyed by methanol, the mixture diluted with water, and extracted with ether. Colourless crystals were deposited which crystallised from benzene as colourless needles (60 mg), m.p. 225–227°, undepressed when mixed with an authentic sample of piperonylic acid.

Identification of compound G: Dihydrocubebin (**9**). The substance crystallised from benzene and also from methanol as colourless cubes, m.p. 112° $[\alpha]_D$ − 42°, R_f 0.28. (Found: C, 66.97; H, 6.21. $C_{20}H_{22}O_6$ requires C, 67.03; H, 6.19%.) (Found: M^+ 358.1416. $C_{20}H_{22}O_6$ requires 358.1416. ν_{max} (Nujol): 3250(OH), 1610(arom.), 930(OCH_2O)cm^{-1}. *m/e* 358(27), 187(11), 136(72), 135(100), 105(12). Labat test positive for OCH_2O. The substance was found to be identical (m.m.p. and NMR) with an authentic sample of (−)-dihydrocubebin.[9]

Reactions of dihydrocubin (**9**)

Acetylation of dihydrocubebin. Dihydrocubebin (100 mg) was heated with acetic anhydride (2.0 ml) and pyridine (1.0 ml) for 4 hr and the product worked up in the usual way. The acetate (**14**) was purified on a column of silica gel and obtained as an uncrystallisable gum (60 g), R_f 0.79[c]. (Found: C, 65.07; H, 5.98. $C_{24}H_{26}O_8$ requires C, 65.15; H, 5.92%). (Found: M' 442.1628. $C_{24}H_{26}O_8$ requires 442.1628.) ν_{max} (Nujol): 1730(OAc), 1600(arom.), 930(OCH_2O)cm^{-1}. *m/e* 442(36), 187(49), 186(16), 174(15), 161(12), 148(14), 136(54), 135(100), 131(16), 105(17).

***Conversion of dihydrocubebin to (−)-hinokinin* (12).** **Dihydrocubebin (200 mg) in anhydrous benzene (50 ml)** was refluxed with silica gel–silver carbonate for 4 hr. The solution was then filtered and the filtrate evaporated under reduced pressure. The residue was chromatographed on a column of silica gel. An uncrystallisable syrup was obtained, R_f 0.84[c], $[\alpha]_D$ − 4.5°. (Found: C, 76.54; H, 5.27. $C_{20}H_{18}O_6$ requires C, 76.79; H, 5.12%). (Found: M^+ 354.1103. $C_{20}H_{18}O_6$ requires 354.1103.) ν_{max} (Nujol): 2880, 1750(γ-lactone), 1600(arom.), 1475, 1430, 1350, 1330 and 925(OCH_2O)cm^{-1}. *m/e* 354(35), 162(10), 136(30), 135(100). Accurate mass measurements *m/e* 192.0782($C_{11}H_{12}O_3$), 162.0680($C_{10}H_{10}O_2$).

Methylation of dihydrocubebin. To a solution of dihydrocubebin (100 mg) in DMF (5.0 ml), methyl iodide (1.5 ml) and freshly precipitated Ag_2O (1.5 g) were added in small portions during a period of 1 hr and shaken at room temperature for 24 hr when the reaction was complete (change in R_f from 0.28 to 0.84). The product (**13**) was purified on a column of silica gel and was obtained as an uncrystallisable syrup, R_f 0.84[c], $[\alpha]_D$ − 7.7°. (Found: C, 68.32; H, 6.81; $C_{22}H_{26}O_6$ requires C, 68.38; H, 6.78%). (Found: M^+ 386.1730. $C_{22}H_{26}O_6$ requires 386.1730). ν_{max} (Nujol): 1600(arom.), 1100 and 925 (OCH_2O)cm^{-1}. *m/e* 386(14), 218(11), 187(32), 174(10), 173(11), 161(12), 136(43), 135(100), 131(11).

Action of methanolic HCl on dihydrocubebin. Dihydrocubebin (100 mg) in methanol (20 ml) was treated with conc HCl (5 drops)

and refluxed for 3 hr when the reaction was found to be complete (change in R_f from 0.28 to 0.83). The reaction mixture was poured into water and extracted with ether. The ether layer was washed with aqueous $NaHCO_3$ and water, and dried over $MgSO_4$. On evaporation of the ether the product (**15**) was obtained as a low melting solid which was purified on a column of silica gel. The pure compound (50 mg) was secured as an uncrystallisable syrup, R_f 0.83[c], $[\alpha]_D$ −45°. (Found: C, 70.52; H, 5.90. $C_{20}H_{20}O_5$ requires C, 70.58; H, 5.92%.) (Found: M^+ 340.1311). ν_{max}(Nujol): 1600(arom.) 1035 (ether) and 925(OCH_2O)cm^{-1}. *m/e* 340(100), 136(87), 106(12).

Oxidation of dihydrocubebin. Dihydrocubebin (100 mg) was dissolved in acetic acid (2 ml), chromium trioxide (100 mg), added, and the mixture left overnight at room temperature. The excess chronium trioxide was destroyed by methanol, the mixture diluted with water and extracted with ether. Upon removal of the ether, colourless crystals were deposited which crystallised from benzene as colourless needles (60 mg), m.p. 225–227°, undepressed when mixed with an authentic sample of piperonylic acid.

Identification of compound H: Diphyllin (**1**)

The substance crystallised as pale yellow needles, m.p. 291° (lit[2] m.p. 291°), R_f 0.51[d]. (Found: C, 66.17; H, 4.43; OCH_3, 16.03. $C_{21}H_{16}O_7$ requires C, 66.32; H, 4.24, OCH_3, 16.23%). ν_{max} (Nujol): 3200 (broad OH), 1730, 1720(γ-lactone), 1600 (arom.) and 930(OCH_2O)cm^{-1}. λ_{max} (EtOH): 360, 330, 295 and 235 nm (log ε 3.90, 4.01, 4.66 and 4.44). *m/e* 380(100), 321(6), 293(11), 175(7). Treatment with Ac_2O/pyridine at 100° for 3 hr gave an acetate, m.p. 235° (lit[2] m.p. 235°). Similarly treatment with DMS/K_2CO_3 in anhydrous acetone at reflux for 10 hr gave a methyl ether, m.p. 262–264° (lit[2] m.p. 263°).

Purification of compounds I and J

The mixture of compounds I and J crystallised from a mixture of benzene, ether and hexane as colourless buttons, m.p. 138–140°, R_f 0.34[d] ν_{max} (Nujol): 3460, 3160(OH), 1750(γ-lactone), 1600 (arom.), 930(OCH_2O)cm^{-1}. The mixture (200 mg) was heated with acetic anhydride (2.0 ml) and pyridine (1.0 ml) on a hot water bath for 6 hr and left overnight at room temperature. Only half of the material was acetylated, as monitored by TLC (change of R_f from 0.34 to 0.75[d]). The reaction product was worked up in the usual way and the acetate of compound I and unacetylated compound J separated on a column of silica gel.

Examination of acetate of compound I: Cleistanthin A acetate

This substance crystallised from a mixture of ether and hexane as colourless needles, m.p. 140°, R_f 0.75[d]. (Found: C, 61.9; H, 5.4. $C_{30}H_{30}O_{12}$ requires C, 61.9; H, 5.2%). ν_{max} (Nujol): 1740(OAc), 1600(arom.), 930(OCH_2O)cm^{-1}. This was found to be identical with cleistanthin A acetate (lit.[2] m.p. 138–140°).

De-acetylation of compound I and identification of compound I: Cleistanthin A (**3**)

The acetate (100 mg) was refluxed with 2% alcoholic KOH solution (20 ml) for 1 hr (change of R_f from 0.75 to 0.34[d]). The solution was neutralised carefully with dil H_2SO_4 and after the usual work up the deacetylated compound crystallised from benzene–hexane as colourless needles, m.p. 135–136°, $[\alpha]_D$ −63.0° (lit[2] m.p. 135–136°, $[\alpha]_D$ −67.2°). (Found: C, 62.18; H, 5.20. $C_{28}H_{28}O_{11}$ requires C, 62.22; H, 5.18%.) ν_{max} (Nujol) 3450(OH), 1750(γ-lactone), 1600(arom), 930(OCH_2O)cm^{-1}. *m/e* 380(100), 292(9). The compound was found to be identical (m.m.p. and NMR) with an authentic sample of cleistanthin A.

Identification of compound J: Cleistanthin D (**24**)

The substance crystallised from methanol as colourless plates, m.p. 199–200°, $[\alpha]_D$ +25°, R_f 0.34[d]. (Found: C, 62.76; H, 5.51. $C_{29}H_{30}O_{11}$ requires C, 62.82; H, 5.42%.) (Found: M^+ 554.1787. $C_{29}H_{30}O_{11}$ requires 554.1787.) ν_{max} (Nujol): 1755(γ-lactone), 1600(arom) 930(OCH_2O)cm^{-1}. λ_{max} (EtOH): 264, 294, 315 and 335 nm(log ε 4.78, 3.98, 4.01 and 3.66). *m/e* 554(9), 381(15), 380(65), 293(11), 175(63), 143(100), 116(15), 115(30), 111(13), 101(94). Accurate mass measurements 380.0907 ($C_{21}H_{16}O_7$), 175.9976($C_8H_{15}O_4$), 143.0712($C_7H_{11}O_3$), 115.0753($C_6H_{11}O_2$).

Hydrolysis of Cleistanthin D (**24**)

A solution of cleistanthin D (250 mg) in CH_2Cl_2 (5 ml) and methanolic HCl (7%, 20 ml) was refluxed at 60° for 6 hr. Upon concentration *in vacuo*, the aglycone separated out on cooling. It crystallised from methanol to yield pale yellow plates (170 mg), m.p. 290°, undepressed by admixture of an authentic sample of diphyllin. Acetate (Ac_2O/phyridine at 100° for 3 hr), m.p. 235° (lit[2] m.p. 235°). Methyl ether (DMS/K_2CO_3 in anhydrous acetone at reflux for 10 hr), m.p. 256° (lit[2] m.p. 263°).

From the above hydrolysate, the solvent was removed under vacuum below 30°, the residue extracted with methanol (2 ml), and the solution passed through a column of dowex-3 (OH) anion exchange resin (500 mg) packed in methanol. Elution of the column with methanol and concentration of the eluate under vacuum, furnished β - methyl - 2,3,5 - tri - O - methyl xyloside as a syrup, $[\alpha]_D$ + 135° (H_2O) (lit[19] + 134°). retention time 2.65 min (column: 15% diethylene glycol succinate on chromosorb, length 2 m, 180°, helium 100 ml/min) (lit retention time 1.6 min, column: 10% carbowax-6000 on 100–200 mesh gas-chrom-A, 125°, argon 100 ml/min). Later, the methyl xyloside was hydrolysed with aqueous HCl (1%, 0.5 ml) and passed through the column of dowex-3(OH) anion exchange resin, which yielded 2,3,5-tri-O-methyl xylose (**22**), $[\alpha]_D$ + 28.5° (H_2O) (lit[19] + 29.5), R_g 0.81 (butanol–ethanol–water 4:1:5v/v).

Preparation of monomethyl ether of cleistanthin A (**21**)

The methyl ether was prepared by shaking cleistanthin A (250 mg) with methyl iodide (2.5 ml) and Ag_2O (1 g) in DMF (5 ml) at room temperature for 10 hr. The product crystallised from ether–petroleum ether as a colourless powder, m.p. 214–215°, (lit[2] m.p. 196–198°) R_f 0.71[d]. *m/e* 554(1), 381(32), 380(100), 293(14), 175(25), 174(15), 143(76), 116(26), 115(22), 111(12), 101(76).

Hydrolysis of O-methyl cleistanthin A (**21**)

A solution of O-methyl cleistanthin A (200 mg) in CH_2Cl_2 (4 ml) and methanolic HCl (7%, 16 ml) was refluxed at 60° for 10 hr. After completion of the reaction and upon concentration of the solution, diphyllin separated, and crystallised from methanol as pale yellow plates (100 mg) m.p. 286–288°.

A filtrate was concentrated at 30° under vacuum and the residue extracted with methanol (2 ml). The solution was passed through a column of dowex-3 (OH) anion exchange resin (500 mg) and eluted with methanol. Upon concentration *in vacuo* the eluate yielded β-methyl-2,3,4-tri-O-methyl xyloside which crystallised from methanol as colourless crystals, m.p. 60–65° (decomp), $[\alpha]_D$ − 74° (lit[19] m.p. 49–50° $[\alpha]_D$ − 81.7°). It showed a retention time of 1.65 min (column: 15% diethylene glycol succinate on Chromosorb, length 2 meters, 180°, helium 100 ml/min) (lit retention time 1.0 min, column: 10% Carbowax-6000 on 100–120 mesh gas-chrom-A, 125°, argon, 100 ml/min). Later, the methyl xyloside was hydrolysed with aqueous HCl (1%, 0.5 ml) and passed through the column of dowex-3 (OH) anion exchange resin, which yielded 2,3,4-tri-O-methyl xylose **23**. $[\alpha]_D$ + 17.5° (H_2O) (lit[19] 18°), R_g 0.93 (n-butanol–ethanol–water 4:1:5v/v).

Identification of compound K. Taiwanin-E 3,4-*di-O-methyl-D-xylopyranoside* (**25**)

Compound K crystallised from methanol as colourless needles, m.p. 210–212°, $[\alpha]_D$ + 11°, R_f 0.59[e]. (Found: C, 61.62; H, 4.82. $C_{27}H_{24}O_{11}$ requires C, 61.83; H, 4.61%.) Molisch test + ve. Labat test + ve. ν_{max} (Nujol) 3460, 3260 (OH), 1750 (γ-lactone), 1610(arom.) and 930(OCH_2O)cm^{-1}. λ_{max}(EtOH) 250, 257, 294, and 346 nm (log ε 4.3, 4.15, 4.6 and 3.9).

Its acetate (acetic anhydride and pyridine at 95° for 3 hr) crystallised from chloroform–methanol (1:1) as colourless needles, m.p. 200–202°, $[\alpha]_D$ + 20°, R_f 0.74[e], (Found: C, 59.42; H, 4.61. $C_{20}H_{26}O_{12}$ requires C, 59.36; H, 4.59% ν_{max} (Nujol) 1755 (γ-lactone), 1735($OCOCH_3$), 1610 (arom) and 930(OCH_2O)cm^{-1}.

Hydrolysis of taiwanin-E 3,4 - *di* - O - *methyl* - D - *xylopyranoside* (**25**)

A solution of compound K (200 mg) in CH_2Cl_2 (2 ml) and methanolic HCl (7%; 8 ml) was refluxed at 60° for 8 hr. After completion of the hydrolysis the solution was concentrated in vacuum and the aglycone separated on cooling. Recrystallisation

from methanol afforded pale yellow crystalline needles, m.p. 305–306°, R_f 0.68[e]. ν_{max} (Nujol) 3200 br (OH), 1740 (γ-lactone), 1600 (arom) and 930 (OCH_2O)cm^{-1}. λ_{max}(EtOH) 249, 257, 303 and 346 nm(log ε 4.3, 4.1, 4.6 *and* 3.9). The substance was identified as taiwanin-E (m.p. IR, NMR).[11,12] Its acetate (Ac_2O-pyridine, 95°, 3 hr) crystallised from methanol as colourless needles, m.p. 310–312°, R_f 0.82[d]. ν_{max} (Nujol) 1750 (γ-lactone), 1730 (acetoxyl), 1610 (arom) and 930 (OCH_2O)cm^{-1}.

The methanolic filtrate from the aglycone was evaporated under vacuum and the residue was treated with aq HCl (1%; 0.5 ml). The solution was passed through a column of Dowex-3 (OH) anion exchange resin (500 mg) packed in methanol. Elution of the column with methanol and concentration of the eluate under vacuum, furnished 3,4-di-O-methylxylose as a syrup, $[\alpha]_D$ + 10.2° (H_2O), R_g 0.81 (butanol–ethanol–water 4:1:5v/v).

Methylation of taiwanin E 3,4-*di-O-methyl-D-xylopyranoside* (25)

A solution of compound K (400 mg) in dimethyl formamide (10 ml) containing silver oxide (2 g) and methyl iodide (4 ml) was stirred for 15 hr. After completion of the methylation as indicated by TLC, the products were worked up in the usual manner and the methyl ether extracted with $CHCl_3$. Upon concentration, it gave a colourless solid which crystallised from chloroform–methanol (1:1) as a colourless powder m.p. 248–250°. R_f 0.68[d]. (Found: C, 62.49; H, 4.88; $C_{28}H_{26}O_{11}$ requires C, 62.44; H, 4.83%.) ν_{max} (Nujol) 1760 (γ-lactone), 1610 (arom) and 930 (OCH_2O)cm^{-1}.

Hydrolysis of O-methyl taiwanin E 3,4-*di-O-methyl-D-xylopyranoside* (25)

A solution of compound K methyl ether (300 mg) in CH_2Cl_2 (8 ml) and methanolic HCl (7%; 18 ml) was refluxed at 60°, for 8 hr. After completion of the hydrolysis and upon concentration of the solution, the aglycone separated out. It crystallised from chloroform–methanol (1:1) to yield pale yellow crystals (170 mg) m.p. 304–306°, unchanged by the admixture of taiwanin E.

From the hydrolysate, the solvent was removed *in vacuo* and the residue was treated with aq. HCl (0.5 ml). It was passed through a column of dowex-3 (OH) anion exchange resin and eluted with methanol. Removal of the solvent under vacuum, yielded 2,3,4-tri-O-methyl xylose, which crystallised from methanol as colourless crystals, m.p. 60–62° (decomp), $[\alpha]_D$ + 17.5°, R_g 0.93.

Bromine oxidation of the sugar of compound K methyl ether

Bromine (0.3 ml) was added to a solution of the sugar (200 mg) in water (5 ml) and the solution heated at 50° for 10 hr. Excess bromine was removed by bubbling air through the solution and the last traces removed by passing SO_2. The solution was extracted with chloroform to yield 2,3,4 - tri - O - methylxylonolactone (80 mg) m.p. 52–55° (decomp), undepressed by the admixture of an authentic sample of 2,3,4 - tri - O - methylxylonolactone. (Found: C, 50.4; H, 7.5; $C_8H_{14}O_5$ requires C, 50.5; H, 7.4%), $[\alpha]_D$ − 3.5° → + 21° (lit[19] m.p. 54° $[\alpha]_D$ − 4 → + 21°).

Identification of compound L. Taiwanin E (11)

Compound L crystallised from methanol as pale yellow needles, m.p. 304–306°, R_f 0.68[e]. (Found: 65.58; H, 3.72%. $C_{20}H_{12}O_7$ requires C, 65.79; H, 3.55%). ν_{max} (Nujol) 3200 br (OH), 1750 (γ-lactone), 1600 (arom.) and 930 (OCH_2O)cm^{-1}. λ_{max} (EtOH) 250, 303, 357 nm (log ε 4.3, 4.6, and 3.9). By comparison with the literature (m.p. IR, NMR)[11,12] the substance was identified as taiwanin-E. Its acetate (acetic anhydride–pyridine, 95°, 3 hr) crystallised from chloroform–methanol (1:1) as colourless needles, m.p. 310–312°, R_f 0.74[d]. ν_{max} (Nujol) 1750 (γ-lactone), 1730 ($OCOCH_3$), 1610 (arom.) and 930 (OCH_2O)cm^{-1}.

Identification of compound M cleistanthin E (26)

The compound crystallised from methanol as colourless buttons, m.p. 178°, $[\alpha]_D$ − 40°, R_f 0.45[e]. (Found: 48.24; H, 5.13. $C_{42}H_{52}O_{20}$ requires C, 48.64; H, 5.03%). ν_{max} ($CHCl_3$) 3400 br (OH), 1760 (γ-lactone), 1610 (arom) and 930 (OCH_2O)cm^{-1}. λ_{max} (EtOH) 266, 298 and 360 nm (log ε 4.75, 4.28 and 4.31). Its acetate (Ac_2O-pyridine at 95°, 3 hr) crystallised from MeOH as colourless needles, m.p. 192°, $[\alpha]_D$ − 42°. R_f 0.70[e]. ν_{max} (Nujol) 1730 (acetoxyl), 1610(arom) and 930 (OCH_2O)cm^{-1}.

Hydrolysis of cleistanthin E (26)

A mixture of cleistanthin-E (100 mg) and methanolic HCl (8%, 16 ml) was refluxed at 65° for 8 hr. After completion of hydrolysis and upon concentration of the solution, the aglycone separated out. It crystallised from chloroform–methanol (1:1) to yield yellow plates (40 mg), m.p. 288–290°, undepressed with authentic diphyllin.

The aglycone acetate (Ac_2O–pyridine at 95° for 3 hr) crystallised from $CHCl_3$–MeOH (1:1), as colourless needles, m.p. 236–238°, undepressed with authentic diphyllin acetate.

The mother liquor was concentrated under vacuum and treated with 1% aq. HCl and the solution passed over a column of Dowex-3 (OH) anion exchange resin. After removal of the solvent from the eluate, the syrup showed 3 clear spots on a paper chromatogram, R_g values 0.81, 0.72 and 0.09 (butanol, ethanol, water 5:1:4). They were identified as β-D-2,3-5-tri-O-methylxylose, β-D-2,3-di-O-methylxylose and β-D-glucose, by comparison with authentic sugars on a co-chromatogram.

Methylation of cleistanthin E (26)

A mixture of cleistanthin-E (100 mg) in dimethyl formamide (2 ml) containing silver oxide (300 mg) and methyl iodide (1 ml) was stirred for 12 hr while monitoring the progress of the reaction by TLC. After completion of the reaction and working up the products as usual, cleistanthin E methyl ether crystallised as colourless buttons., m.p. 140–142°, $[\alpha]_D$ − 36°, R_f 0.71[d]. ν_{max} (Nujol) 1755 (γ-lactone), 1610 (arom) and 930 cm^{-1}.

Hydrolysis of cleistanthin E-methyl ether

A mixture of cleistanthin E methyl ether (50 mg) and 7% methanolic HCl (8 ml) was refluxed at 60° for 4 hr. After concentration of the solution, the aglycone was filtered off and the mother liquor was passed over a column of Dowex-3 (OH) anion exchange resin. After removal of the solvent under vacuum, the syrup was examined on a paper chromatogram (butanol, ethanol, water; 5:1:4). The chromatogram showed a new spot at R_g 0.83, in addition to the usual spots corresponding to 2,3,5-tri-O-methyl xylose (0.81) and 2,3-di-O-methyl xylose (0.72). The sugar with R_g 0.83 was identified as 2,3,6-tri-O-methyl-glucose by comparison with an authentic sample on a co-chromatogram.

Partial hydrolysis of cleistanthin E (26)

A solution of cleistanthin E (100 mg) in aqueous methanolic H_2SO_4 (0.05N, 20 ml) was refluxed at 60°. After 12 hr, the reaction mixture was cooled to 30° and the solid which separated was filtered off. The mother liquor was passed over a column of Dowex-3 (OH) anion exchange resin. After removal of the solvent, the syrup showed two spots on a paper chromatogram, R_g 0.81, 0.71, which were identified as 2,3,5-tri-O-methyl xylose and 2,3-di-O-methyl xylose by comparison with authentic sugars on a co-chromatogram.

Identification of compound N cleistanthin C (5)

Compound N crystallised from chloroform–methanol as colourless plates m.p. 220–221°, $[\alpha]_D$ − 33°, R_f 0.22[e]. (Found: C, 55.18; H, 5.67. $C_{34}H_{38}O_{16}$, $2H_2O$ requires C, 55.29, H, 5.69%). ν_{max} (Nujol) 3350 (br OH), 1770 (γ-lactone), 1620 (arom) and 930 (OCH_2O)cm^{-1}. λ_{max} (EtOH) 350, 297 and 265 nm (log ε 4.31, 4.32 and 4.79). It was identified as cleistanthin C (m.m.p. and IR)[5] by comparison with an authentic sample. Acetate (Ac_2O + pyridine) m.p. 150–152° (lit[5] m.p. 152–154°). Compound N methyl ether (Ag_2O + CH_3I + DMF), m.p. 116–118°, $[\alpha]_D$ + 21.5°.

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