METABOLITES OF MICROORGANISMS. 242†

PYRIDINDOLOL GLUCOSIDES FROM *STREPTOMYCES PARVULUS*

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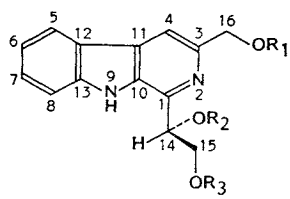
From a new strain of Streptomyces, *Streptomyces parvulus*, strain Tü 2480 three glucosides of the alkaloid pyridindolol were isolated. The structure elucidation is based on spectroscopic investigations and degradation to pyridindolol and α,D-methyl glucoside.

From cultures of an actinomycete, strain Tü 2480, we have recently isolated (*E*)-3-(1*H*-pyrrol-3-yl)propenic acid and its amide[2].

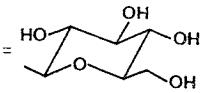
Late chromatographic fractions of the same extracts and particularly a precipitate nearly insoluble in ethyl acetate[2] contained four compounds showing an intense blue fluorescence upon irradiation with UV light (254 or 366 nm) on TLC plates. The compounds could be separated by repeated chromatographic procedures, and gave colorless crystals or powders upon recrystallization form MeOH - EtOAc. They were provisionally named F_1, F_2, F_3 and F_4 according to decreasing Rf values in TLC (0.73, 0.36, 0.29, 0.23; $CHCl_3$ - MeOH - 25% NH_3 (6:5:1), Silica gel Merck F_{254}). Compound F_1 could be identified by its analytical and spectroscopic properties with pyridindolol (**1**), a metabolite isolated by UMEZAWA and his co-workers[3,4] from a *Streptomyces* strain, and showing galactosidase inhibiting activity. The compounds F_2, F_3 and F_4 are isomers of the molecular formula $C_{20}H_{24}N_2O_8$. The UV spectra are the same as that of pyridindolol itself, indicating that pyridindolol is a common constituent of the three new metabolites. All give hexaacetates on acetylation showing molecular ions with *m/z* 672 ($C_{32}H_{36}N_2O_{14}^+$) in the mass spectra.

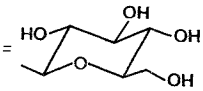
The molecular formulae $C_{20}H_{24}N_2O_8$ of F_2, F_3 and F_4 are consistent with pyridindolol hexoside structures. In agreement with this are the ^{13}C NMR spectra of the acetylation products of F_2, F_3 and F_4 (Table 2), which show in addition to the carbon signals of pyridindolol an acetal carbon signal near 100 ppm and 4 CH and 1 CH_2 signals in the region of alcoholic carbon resonances. The nature of the sugar moieties was elucidated by methanolysis. The compounds F_2, F_3 and F_4 gave all pyridindolol (**1**) as the aglycone, identified by mp (166°C), TLC, ^1H NMR and specific rotation. Surprisingly also the sugar components of the three glycosides proved to be identical: D-Glucose. The methyl glycosides (anomeric mixtures) obtained by methanolysis were acetylated and then separated by preparative TLC. In all cases α,D-methyl glucoside tetraacetate was obtained and identified by comparison with a sample prepared from authentic D-glucose by TLC, ^1H NMR, $[\alpha]_D$ ($[\alpha]_D^{25}$ +134° in $CHCl_3$)[4]. From pyridindolol glucoside F_2 also the anomeric β,D-methyl glucoside tetraacetate

† Preceding communication see ref 1.



Tü 2480 F_1 $R_1 = R_2 = R_3 = H$

Tü 2480 F_2 $R_1 = R_3 = H$ $R_2 =$ 

Tü 2480 F_3 $R_1 = R_2 = H$ $R_3 =$ 

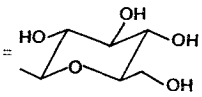
Tü 2480 F_4 $R_1 =$  $R_2 = R_3 = H$

Table 1. ^{13}C NMR spectra of Tü 2480 F_1 and F_2 (DMSO-d_6, 25 MHz).

Assignments	F_1 (pyridindolol)	F_2
Aromatic carbon atoms	148.9 s[a]	149.4 s
	144.4 s	141.4 s
	140.8 s	140.9 s
	132.3 s	132.9 s
	129.0 s	129.5 s
	127.7 d	127.9 d
	121.2 d	121.4 d
	120.4 s	120.4 s
	118.8 d	119.1 d
	112.2 d	112.0 d
	109.8 d	110.3 d
Pyridindolol side chains	74.4 d	73.8 d
	65.4 t	64.5 t
	64.5 t	64.4 t
Sugar moiety		101.2 d
		81.5 d
		77.1 d
		76.4 d
		70.2 d
		61.1 t

[a] Multiplicities in off-resonance spectra.

Table 2. ^{13}C NMR spectra of acetyl derivatives ($CDCl_3$, 25 MHz).

Assignments	F_2	F_3	F_4
Aromatic carbon atoms	143.9 s	143.8 s	146.2 s
	140.8 s	141.3 s	140.9 s
	140.2 s	139.2 s	137.4 s
	133.4 s	134.1 s	133.7 s
	130.7 s	130.9 s	131.2 s
	129.0 d	128.8 d	129.0 d
	121.7 d	121.6 d	121.7 d
	120.8 s	121.3 s	121.6 s
	120.3 d	120.1 d	120.5 d
	114.1 d	114.3 d	113.4 d
	112.0 d	112.4 d	112.0 d
Pyridindolol side chains	72.7 d	72.5 d	72.2 d
	67.7 t	70.7 t	72.5 t
	65.9 t	67.8 t	64.6 t
Sugar moieties	100.8 d	100.2 d	100.3 d
	82.9 d	74.5 d	73.1 d
	72.2 d	72.0 d	72.1 d
	71.6 d	71.7 d	71.7 d
	68.5 d	68.4 d	68.7 d
	62.0 t	61.9 t	62.1 t
Acetyl groups	170.9 s	171.0 s	171.4 s
	170.7 s	170.7 s	171.0 s
	170.5 s, 2C	170.5 s	170.7 s
		170.1 s	170.3 s
	170.0 s	170.0 s	169.5 s, 2C
	169.5 s	169.4 s	
	21.2 q	21.2 q	21.2 q
	20.8 q	21.0 q	20.9 q
	20.7 q	20.8 q, 2C	20.8 q, 2C
	20.6 q, 2C	20.7 q, 2C	20.7 q, 2C
	20.2 q		

was obtained in pure form and identified by comparison with an authentic sample; $[\alpha]_D^{22}$ $-17°$ (c 0.30, $CHCl_3$)[5].

The ^1H NMR spectra of the three pyridindolol glucosides show signals for the protons at C(1′) of the sugar component at 4.37±0.02 ppm, all being doublets with coupling constants J=7.3~7.8 Hz, which clearly indicate that all three compounds are β-glucosides. F_2, F_3 and F_4 must therefore be positional isomers.

The positions of the sugar moieties could nicely be deduced from the ^1H NMR spectra of the acetyl derivatives (Table 3). The protons at C(14) show chemical shifts near 6.5 ppm in F_1, F_3 and F_4 acetate, whereas in F_2 acetate the corresponding proton gives a resonance at 5.4 ppm (5.33 ppm in the non acetylated compound). The glucose residue is thus linked to position 14 in F_2, to which structural formula **2** is assigned. The protons at C(15) give signals at 4.2 and 4.5 ppm in F_3 acetate, but near 4.8 ppm in F_1 and F_4 acetates, at 4.5~4.7 in F_2 acetate. Structure **3** with the glucose residue in position 15 is therefore assigned to glucoside F_3.

For the compound F_4 there remains structure **4** with the glucose residue at position 16. This is confirmed by the ^1H NMR spectrum of its acetate (Table 3), in which the signals of the protons at C(16) must be a part of the heap of signals at 5.1~5.3 ppm, whereas in F_2 and F_3 acetates the corresponding signals are found as an AB group or as a singlet near 5.35 ppm as in pyridindolol acetate. Although the acetylation effect is small in this particular case, the spectra in Table 3 are in best agree-

Table 3. ^1H NMR spectra of acetyl derivatives of pyridindolol and its glucosides ($CDCl_3$, 300 MHz).

Assignments	^1H NMR (ppm)				
	F_1	F_2	F_3	F_4	Tetra-*O*-acetyl-β, D-methyl glucoside
4-H	8.00 s	7.99 s	8.01 s	8.02 s	—
5-H	7.55 2H, m	7.58 2H, m	7.69 br d	7.55 2H, m	—
6-H			7.57 ddd		
7-H	7.29 ddd	7.28 ddd	7.29 ddd	7.29 ddd	—
8-H	8.10 dd	8.11 br d	8.12 br d	8.09 br d	—
NH	9.17 s	8.91 s	9.37 s	9.12 s	—
14-H	6.55 dd	5.40 dd	6.36 t	6.53 dd	—
15-H_a	4.87 dd	4.67 dd	4.48 dd	4.87 dd	—
15-H_b	4.80 dd	4.49 dd	4.20 dd	4.76 dd	—
16-H_a	5.37 2H, s	5.36 d	5.38 2H, s	5.1~5.3 4H[a], m	—
16-H_b		5.34 d			
1′-H	—	4.72 d	4.57 d	4.77 d	4.43 d
2′-H	—	5.1~5.3 3H[a], m	4.93 t	5.1~5.3 4H[a], m	4.98 dd
3′-H	—		4.96 t	4.88 t	5.08 t
4′-H	—		5.20 t	5.1~5.3 4H[a], m	5.20 t
5′-H	—	3.74 ddd	3.69 ddd	3.76 ddd	3.70 ddd
6′-H_a	—	4.32 dd	4.19 dd	4.31 dd	4.28 dd
6′-H_b	—	4.18 dd	4.06 dd	4.17 dd	4.15 dd
OCH_3	—	—	—	—	3.50 3H, s
$COCH_3$	2.19 3H, s	2.18 3H, s	2.17 3H, s	2.19 3H, s	2.08 3H, s
	2.17 3H, s	2.15 3H, s	2.12 3H, s	2.08 3H, s	2.04 3H, s
	2.08 3H, s	2.02 3H, s	2.01 3H, s	2.07 3H, s	2.02 3H, s
		1.97 3H, s	2.00 3H, s	2.03 3H, s	2.00 3H, s
		1.92 3H, s	1.99 3H, s	2.01 3H, s	—
		1.34 3H, s	1.95 3H, s	1.99 3H, s	—

[a] Overlapping signals.

Table 3. (Continued)

	Coupling constants (Hz)				
	F_1	F_2	F_3	F_4	Tetra-*O*-acetyl-β, D-methyl glucoside
$J_{5,6}$	8.0	7.9	7.9	7.9	—
$J_{5,7}$	0.7	0	1.1	0	—
$J_{6,7}$	5.5	6.0	7.1	5.4	—
$J_{6,8}$	2.5	2.0	1.0	2.6	—
$J_{7,8}$	8.1	?	8.2	8.0	—
$J_{14,15a}$	7.9	6.0	4.5	8.1	—
$J_{14,15b}$	3.5	4.0	4.5	3.3	—
$J_{15a,15b}$	12.3	11.8	10.5	12.3	—
$J_{16a,16b}$	0	12.1	0	?[a]	—
$J_{1',2'}$	—	7.8	7.8	7.2	7.9
$J_{2',3'}$	—	?[a]	9.5	?[a]	9.5
$J_{3',4'}$	—	?[a]	9.5	?[a]	9.5
$J_{4',5'}$	—	9.8	10.0	9.8	9.8
$J_{5',6'a}$	—	5.2	4.9	4.7	4.7
$J_{5',6'b}$	—	2.4	2.4	2.4	2.5
$J_{6'a,6'b}$	—	12.2	12.3	12.3	12.3

[a] Overlapping signals.

ment with the assigned structures.

A striking spectral detail is the highfield position of one of the acetyl signals in the spectrum of F_2 acetate, 1.34 ppm (Table 3). Model studies show that in all possible conformations of a pyridindolol 14-glucoside hexaacetate one acetyl group is situated below the plane of the aromatic system. This unusual chemical shift is therefore an effect of the ring current.

It is interesting that all 3 hydroxyl groups of pyridindolol can be involved in a glucoside bond. However we never observed the presence of di- or triglucosides in the extracts of strain Tü 2480.

In this connection the recent isolation of the harmane alkaloid flazin[6], from soy sauce deserves some consideration. It seems to be not unlikely that compounds of this type from soy bean meal, which is a constituent of the Nutrient media, serve as biogenetic precursors of pyridindolol and its glucosides.

Experimental

Isolation of Pyridindolol and its Glucosides

A precipitate nearly insoluble in EtOAc from a 100-liter culture[2] was extracted 7 times with MeOH and filtered. The filtrate gave 160 g of brown tar upon evaporation *in vacuo*, containing the F components. The insoluble part (68 g) did not contain further F substances and was discarded.

A part of the F mixture (40 g) was separated by flash chromatography on silica gel (9×23 cm, eluents EtOAc - MeOH - H_2O, 75 : 10 : 7, 75 : 15 : 10 and 45 : 15 : 10) into 6.5 g of crude F_1 and 10 g of a mixture of F_2, F_3 and F_4. The F_1 fraction was again chromatographed on silica gel with $CHCl_3$ - MeOH - NH_3[†], 13 : 5 : 1 to yield 3.0 g of F_1 and 1.9 g of $F_2 \sim F_4$. The F_1 fraction was further purified on a column of Sephadex LH-20 with MeOH and then by slow crystallization from MeOH - EtOAc; 2.0 g of colorless needles, mp 165°C, were obtained, $[\alpha]_D^{25}$ −48° (*c* 1.5, MeOH). All spectra (UV, IR, ^1H NMR and ^{13}C NMR) were in best agreement with those reported for pyridindolol (**1**)[3,4]; ^{13}C NMR Table 1.

The $F_2 \sim F_4$ fraction (11.9 g) was chromatographed on silica gel (9×23 cm, flash chromatography;

[†] 25% NH_3 in H_2O.

$CHCl_3$ - MeOH - NH_3, 6:5:1) to yield 0.9 g of F_1, 3.9 g of F_2, 1.4 g of F_3 and 5.2 g of a mixture of F_3 and F_4. The latter was further separated with $CHCl_3$ - MeOH - NH_3, 6:6:1, 5:6:1, 3:6:1 and 1:6:1 (subsequent eluents), 0.6 g F_3 and 0.2 g F_4 were obtained in pure form. After similar separations of additional crude material and mixture fractions a total of 4.9 g F_2, 2.5 g F_3 and 2.4 g F_4 were isolated from 100 liters of culture. The compounds were further purified on columns of Sephadex LH-20 (MeOH).

Pyridindolol 14-β,D-Glucoside (**2**) from F_2 Fractions: After recrystallization from MeOH - EtOAc colorless clusters of needles: MP 228~231°C (dec); Rf 0.34 (TLC, EtOAc - MeOH - H_2O, 75:10:7); $[\alpha]_D^{20}$ −100° (*c* 0.5, MeOH); UV λ_{max}^{EtOH} nm (log ε) 356 (3.66), 345 (3.66), 291 (4.18), 237 (4.51), 216 (4.05); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.25 (1H, s, exchangeable), 8.22 (1H, d, J=7.8 Hz), 8.08 (1H, s), 7.59 (1H, d, J=8.1 Hz), 7.52 (1H, ddd, J=8.1, 7.0 and 1.1 Hz), 7.21 (1H, ddd, J=7.8, 7.0 and 1.1 Hz), 6.07 (1H, br d, J=3.7 Hz, exchangeable), 5.33 (1H, t, J=5.8 Hz), 5.31 (1H, t, J=4.2 Hz, exchangeable), 5.09 (1H, br d, J=4.0 Hz, exchangeable), 4.92 (1H, br d, J=3.3 Hz, exchangeable), 4.77 (1H, t, J=6.0 Hz, exchangeable), 4.70 (2H, d, J=5.6 Hz; s after exchange with D_2O), 4.65 (1H, t, J=5.6 Hz, exchangeable), 4.39 (1H, d, J=7.7 Hz), 3.98 (2H m; AB after exchange), 3.74 (1H, dd, J=11.4 and 5.3 Hz; after exchange: d, J=11.4 Hz), 3.50 (1H, m), 3.14 (4H, m); ^{13}C NMR Table 1; electron impact mass spectra (EI-MS) *m/z* (relative intensity %) 420 (1, M^+), 402 (2), 241 (13), 240 (69), 239 (50), 225 (15), 224 (78), 223 (32), 212 (11), 211 (23), 196 (12), 195 (19), 194 (16), 193 (15), 182 (19), 181 (19), 180 (42), 179 (25), 169 (18), 168 (15), 167 (17), 154 (15), 140 (23), 136 (53), 127 (18), 115 (20), 114 (12), 98 (13), 97 (12), 81 (19), 73 (27), 69 (14), 61 (15), 60 (41), 57 (33), 55 (22), 54 (15), 45 (23), 44 (28), 43 (100), 42 (22).

Anal Calcd for $C_{20}H_{24}N_2O_8 \cdot \frac{1}{3}H_2O$: C 56.33, H 5.83, N 6.57.
Found: C 56.56, H 5.81, N 6.49.

Pyridindolol 15-β,D-Glucoside (**3**) from F_3 Fractions: Colorless amorphous powder; Rf 0.35 (TLC, $CHCl_3$ - MeOH - NH_3, 5:6:1); Rf 0.37 (TLC, Me_2CO - MeOH - NH_3, 4:6:1); $[\alpha]_D^{20}$ −90° (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.15 (br s, exchangeable), 8.22 (1H, d, J=7.8 Hz), 8.11 (1H, s), 7.68 (1H, d, J=8.2 Hz), 7.53 (1H, t, J=7.6 Hz), 7.22 (1H, t, J=7.5 Hz), 5.76 (1H, br s, exchangeable), 5.36 (1H, br s, exchangeable), 5.30 (1H, m; after exchange with D_2O: t, J=5.6 Hz), 5.11 (1H, br s, exchangeable), 4.91 (1H, br s, exchangeable), 4.73 (2H, s), 4.40 (2H, br s, exchangeable), 4.35 (1H, d, J=7.8 Hz), 4.10 (1H, dd, J=10.8 and 6.3 Hz), 3.99 (1H, dd, J=10.8 and 5.0 Hz), 3.72 (1H, d, J=11.0 Hz), 3.49 (1H, dd, J=11.6 and 5.5 Hz), 3.19 (2H, m), 3.09 (1H, m; after exchange: t, J=9.2 Hz), 2.93 (1H, t, J=8.4 Hz); EI-MS *m/z* (relative intensity %) 420 (0.3, M^+), 402 (0.4), 358 (1), 276 (4), 258 (14), 257 (2), 241 (6), 240 (8), 227 (3), 224 (9), 223 (6), 216 (12), 215 (80), 212 (6), 197 (10), 195 (8), 182 (6), 170 (21), 169 (100), 168 (24), 150 (14), 140 (9), 137 (9), 132 (21), 130 (11), 121 (7), 115 (13), 107 (8), 106 (41), 91 (7), 85 (8), 84.5 (6), 77 (15), 73 (13), 65 (7), 61 (9), 60 (7), 57 (7), 55 (7), 51 (7), 45 (7), 44 (31), 43 (20), 41 (8), 39 (9), 31 (17), 29 (9), 28 (25), 18 (21).

Pyridindolol 16-β,D-Glucoside (**4**) from F_4 Fractions: A fraction enriched in F_4 (2.4 g) was repeatedly chromatographed on silica gel (5×21 cm) with $CHCl_3$ - MeOH - NH_3 (3:6:1) and on Sephadex LH-20 with MeOH as eluent. By evaporation *in vacuo* a colorless amorphous powder of pure F_4 was obtained. Rf 0.35 (TLC, $CHCl_3$ - MeOH - NH_3, 3:6:1); $[\alpha]_D^{22}$ −80° (*c* 0.5, MeOH); UV λ_{max}^{EtOH} nm (log ε) 351 (3.66), 340 (3.16), 289 (4.17), 237 (4.55), 214 (4.07); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.81 (1H, s, exchangeable), 8.20 (1H, s), 8.16 (1H, d, J=7.8 Hz), 7.67 (1H, d, J=8.2 Hz), 7.50 (1H, t, J=7.7 Hz), 7.21 (1H, t, J=7.5 Hz), 5.67 (1H, d, J=4.8 Hz, exchangeable), 5.23 (1H, br d, J=3.9 Hz, exchangeable), 5.04 (1H, br m), 5.03 (1H, d, J=12.5 Hz), 4.93 (1H, br d, J=3.9 Hz, exchangeable), 4.88 (1H, br d, J=4.3 Hz, exchangeable), 4.80 (1H, d, J=12.5 Hz), 4.73 (1H, br t, J=5.2 Hz, exchangeable), 4.55 (1H, t, J=5.8 Hz, exchangeable), 4.37 (1H, d, J=7.3 Hz), 3.84 (2H, m), 3.74 (1H, dd, J=11.8 and 5.6 Hz), 3.51 (1H, m), 3.16 (4H, m); EI-MS *m/z* (relative intensity %) 402 (1, $M^+ - H_2O$), 239 (5), 225 (18), 224 (100), 223 (49), 211 (8), 210 (16), 209 (9), 196 (25), 195 (27), 182 (13), 181 (13), 180 (7), 179 (9), 168 (4), 167 (4), 154 (9), 127 (8), 73 (7), 61 (8), 60 (11), 57 (7), 55 (5), 43 (26).

Acetylation of Pyridindolol and its Glucosides

From 54 mg of F_1 (pyridindolol, **1**) in 5 ml pyridine and 3 ml Ac_2O (room temp, 16 hours) triacetyl pyridindolol was prepared. After chromatography on silica gel ($CHCl_3$ - EtOAc, 2:1) and recry-

stallization from ether 68 mg of colorless crystals were obtained: MP 90°C; $[\alpha]_D^{25}$ −27° (*c* 1.1, $CHCl_3$); UV λ_{max}^{EtOH} nm (log ε) 352 (3.64), 342 (3.63), 291 (4.16), 238 (4.52), 214 (4.40); IR, ^1H NMR and MS in agreement with spectra reported in the literature[3,4]; ^1H NMR Table 3.

Anal Calcd for $C_{20}H_{20}N_2O_6$ (384.39): C 62.49, H 5.24, N 7.29.
Found: C 62.36, H 5.22, N 7.19.

The acetylation product prepared from 132 mg F_3 in 3 ml pyridine and 6 ml Ac_2O in analogous manner was purified by chromatography on silica gel ($CHCl_3$ - EtOAc, 5:9). The homogenous fractions gave 101 mg of a colorless amorphous powder; Rf 0.32 (TLC, $CHCl_3$ - EtOAc, 5:9); $[\alpha]_D^{25}$ −89° (*c* 0.49, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3400 (br m), 3000 (w), 2950 (w), 2875 (w), 1752 (s), 1743 (s), 1629 (m), 1602 (w), 1570 (w), 1496 (m), 1468 (w), 1454 (m), 1430 (w), 1369 (s), 1325 (w), 1235 (s), 1173 (w), 1125 (w), 1043 (s), 961 (w), 810 (w); ^1H NMR Table 3; ^{13}C NMR Table 2; EI-MS *m/z* (relative intensity %) 672 (5, M^+), 629 (3), 613 (2), 331 (5), 325 (18), 324 (8), 281 (6), 267 (7), 265 (8), 239 (5), 224 (6), 223 (29), 222 (7), 209 (10), 207 (5), 195 (11), 169 (75), 145 (7), 139 (6), 133 (12), 132 (8), 127 (16), 115 (10), 109 (56), 103 (7), 98 (6), 97 (7), 85 (4), 81 (9), 73 (4), 69 (5), 60 (18), 57 (7), 45 (18), 43 (100), 28 (7), 18 (7).

In similar manner 38 mg F_4 was acetylated and yielded, after repeated chromatography and preparative TLC 20 mg of homogenous hexaacetate; Rf 0.34 (TLC, $CHCl_3$ - EtOAc, 3:4); $[\alpha]_D^{22}$ −70° (*c* 0.5, $CHCl_3$); ^1H NMR Table 3; ^{13}C NMR Table 2; EI-MS *m/z* (relative intensity %) 672 (0.5, M^+), 613 (1), 325 (1), 265 (1), 223 (3), 207 (2), 195 (5), 169 (2), 154 (3), 139 (3), 127 (6), 115 (5), 109 (11), 102 (4), 98 (4), 97 (7), 85 (3), 81 (5), 73 (4), 69 (5), 60 (11), 57 (2), 55 (3), 45 (20), 44 (7), 43 (100), 42 (9), 29 (6), 28 (3), 15 (10).

The acetylation of 163 mg F_2 in 4 ml pyridine and 7 ml Ac_2O was carried out at 100°C (70 minutes). The crude product was chromatographed on silica gel ($CHCl_3$ - EtOAc, 1:1) and on Sephadex LH-20 (MeOH). The homogenous product (129 mg) was a colorless amorphous powder; $[\alpha]_D^{25}$ −103° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3440 (m), 3020 (w), 2995 (w), 2945 (m), 1752 (s), 1743 (s), 1637 (m), 1601 (w), 1569 (w), 1494 (m), 1469 (w), 1453 (m), 1356 (s), 1319 (w), 1235 (s), 1150 (w), 1211 (w), 1047 (s), 919 (w); ^1H NMR Table 3; ^{13}C NMR Table 2; EI-MS *m/z* (relative intensity %) 672 (3, M^+), 629 (1), 613 (1), 331 (8), 326 (11), 325 (23), 267 (25), 266 (31), 265 (13), 239 (6), 225 (6), 224 (8), 223 (40), 222 (7), 207 (11), 202 (14), 195 (10), 181 (2), 169 (54), 127 (10), 109 (37), 97 (10), 87 (40), 83 (11), 73 (8), 71 (12), 69 (15), 60 (12), 57 (21), 55 (14), 43 (100), 41 (12), 28 (8), 18 (25).

Hydrolysis of Pyridindolol Glucoside F_3

A solution of 160 mg F_3 in 10 ml 3 M HCl was stirred 21 hours at 100°C. The ice cooled solution was neutralized with aq NaOH and evaporated *in vacuo*. The crude mixture was separated on silica gel (2×21 cm, flash chromatography, $CHCl_3$ - MeOH - NH_3, 4:6:1) into the aglycone and a sugar fraction. The aglycone fraction gave after further purification (silica gel, $CHCl_3$ - MeOH - NH_3, 15:5:1) and recrystallization 31 mg pyridindolol, mp 166°C, identified by TLC, IR and ^1H NMR; $[\alpha]_D^{25}$ −48° (*c* 1.45, MeOH).

The more polar sugar fraction was dried under high *vacuum* and then acetylated with 3 ml pyridine and 6 ml Ac_2O (15 hours, room temp). However, the mixture of anomeric glucose pentaacetates (95 mg) could not easily be separated by preparative TLC. It was, therefore, methanolized (HCl solution prepared by dissolution of 5 ml AcCl in 8 ml abs MeOH) at 75°C (14 hours, under N_2). The solution was then neutralized with NH_3 and evaporated to dryness. The residue was extracted with MeOH and filtered from NH_4Cl. The filtrate was evaporated and dried under reduced pressure. After acetylation with 3 ml pyridine and 6 ml Ac_2O the anomeric methyl D-glucoside tetraacetates were separated by repeated preparative TLC with $CHCl_3$ - EtOAc (14:1) and CH_2Cl_2 - Me_2CO (19:1) as the solvents. From the upper zone 5 mg of pure α-methyl D-glucoside tetraacetate was obtained, $[\alpha]_D^{25}$ +131° (*c* 0.245, $CHCl_3$), according to TLC and ^1H NMR identical with a sample prepared from authentic D-glucose.

Methanolysis of Pyridindolol Glucosides F_2 and F_4

A solution of 260 mg F_2 in 70 ml 3 M HCl in abs MeOH was stirred at 60°C. After 6 days the reaction was complete (TLC). The solution was neutralized with NH_3 and evaporated in high *vacuum*

to dryness. The residue was extracted with MeOH and the filtered solution again evaporated and dried. Chromatography on silica gel (2×24 cm, $CHCl_3$ - MeOH - NH_3, 5:5:1, flash method) gave 26 mg pyridindolol (after chromatography on Sephadex LH-20 and recrystallization), identified by mixed mp, IR and ^1H NMR; $[\alpha]_D^{25}$ −48° (*c* 0.60, MeOH) and a mixture of the anomeric methyl D-glucosides (87 mg) after chromatography on Sephadex LH-20 with MeOH. 37 mg of this material was acetylated in 7 ml pyridine and 4 ml Ac_2O 16 hours at room temp. The brownish product was purified by flash chromatography ($CHCl_3$ - EtOAc, 14:1). By repeated preparative TLC with CH_2Cl_2 - Me_2CO (19:1) and $CHCl_3$ - EtOAc (14:1) 14 mg of the pure α-anomer (Rf 0.31) was obtained as a colorless liquid, $[\alpha]_D^{25}$ +133° (*c* 1.07, $CHCl_3$), identified by TLC and ^1H NMR. From a second zone 6 mg of β-methyl D-glucoside tetraacetate (Rf 0.27) was obtained, $[\alpha]_D^{22}$ −17° (*c* 0.30, $CHCl_3$). The identity was shown by comparison with a sample prepared from commercial D-glucose (TLC and ^1H NMR).

By the same procedure from 75 mg F_4 25 mg of pyridindolol and a mixture of anomeric methyl D-glucosides were obtained. The aglycone was identified after acetylation (22 mg crystals, mp 89°C, $[\alpha]_D^{25}$ −23° (*c* 1.10, $CHCl_3$)) by TLC, IR and ^1H NMR. The methyl glucoside fraction was acetylated as above, and the acetates were separated by preparative TLC (see above). The major α-anomer (14 mg) was still contaminated with *ca.* 10% of the β-glucoside; $[\alpha]_D^{25}$ +112° (*c* 0.70, $CHCl_3$). The identity with α-methyl D-glucoside tetraacetate followed from the ^1H NMR signals of the main component (*ca.* 90%) and from Rf (TLC). The β-anomer was impure and was not identified except by TLC.

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