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Note

Synthesis of the glucuronide of Carazolol

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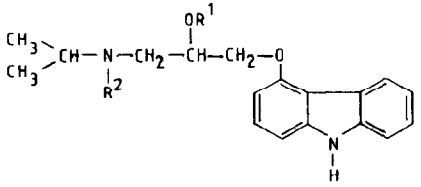
Carazolol [1-(9*H*-carbazol-4-yloxy)-3-isopropylamino-2-propanol] is a lipophilic beta-blocking agent used in humans and animals[1,2] as the racemate, although only the *S*-(−) enantiomer is active. Carazolol prevents metabolic disorders in pigs due to stress, *e.g.*, during transportation. Lipophilic beta-blocking agents[3,4] are extensively metabolised. Carazolol can be determined in tissues from pigs[5], but its glucuronide, which should be a major metabolite[6], has not been identified hitherto[5,7]. We now describe the synthesis of the glucuronides of the (*R*)- and (*S*)-forms of Carazolol.

Treatment of racemic Carazolol (**1**) with di-*tert*-butyl carbonate in aqueous alkali–1,4-dioxane[8] gave 78% of the *N*-*tert*-butoxycarbonyl derivative **2** and 12% of the di-*tert*-butoxycarbonyl derivative **3**. Reaction of **2** with methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl bromide)uronate[9] in toluene in the presence of silver carbonate[10,11] and molecular sieve (4Å) gave 82% of a 1:1.1 mixture of the *R*-(+) (**4**) and *S*-(−) (**5**) derivatives as indicated by the ^{1}H-n.m.r. data.



1 $R^1 = R^2 = H$
2 $R^1 = H, R^2 = Boc$
3 $R^1 = R^2 = Boc$

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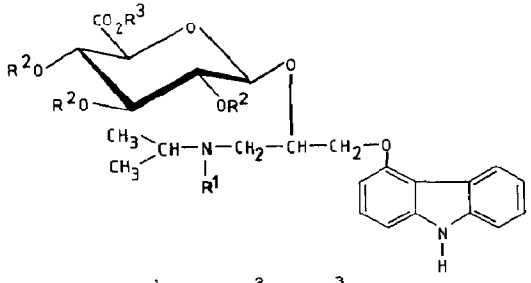
Treatment of **4** and **5** severally with trifluoroacetic acid and chromatography of the products gave the *R*-(+)- (**6**) and *S*-(−)-isopropylaminoprop-2-yl glucuronide (**7**) derivatives. The β configuration of the glycosidic linkages in **6** and **7** was apparent from the ^{1}H-n.m.r. data for the H-1 resonances (**6**, δ 5.07, $J_{1'',2''}$ 7.8 Hz; **7**, δ 4.99, $J_{1'',2''}$ 7.9 Hz). The anomers were not formed, because of the neighboring-group effect[12] of AcO-2''. The ^{1}H-n.m.r. spectra of **6** and **7** were similar, except for a marked shift of one AcO resonance in **7** to high field (1.48 p.p.m.) because of the diamagnetic effect of the aromatic system.

Treatment of **6** and **7** with methanolic 0.05M sodium methoxide for 12 h at room temperature gave the sodium salts of the *R*-(+) (**8**) and *S*-(−) glucuronide (**9**), respectively, in quantitative yield. The structures of **8** and **9** indicated by the ^{1}H-n.m.r. and mass spectra were confirmed by a synthesis from *R*-(+)-Carazolol (supplied by Boehringer Mannheim) and by hydrolysis of **8** and **9** with β-D-glucuronidase to give the known (*R*) and (*S*) enantiomers of Carazolol.



4, **5** R^1 = Boc, R^2 = Ac, R^3 = Me
6, **7** R^1 = H, R^2 = Ac, R^3 = Me
8, **9** R^1 = R^2 = R^3 = H

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP 61 apparatus and are uncorrected. Optical rotations were measured at 22 ±1° with a Perkin–Elmer 241 polarimeter. ^{1}H-N.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (HOD 4.64 p.p.m.) with Bruker WM 270 and WM 400 instruments. Protons from amino and hydroxyl groups were exchanged with deuterium, except where stated otherwise. Mass spectra were determined on a Finnigan MAT 8230 spectrometer, using the direct inlet at 150° (EI; 70 eV).

The purity of products was determined by t.l.c. on Silica Gel F_{254} (Merck) with detection by u.v. light or by charring with sulphuric acid. Column chromatography was performed on Silica Gel 60 (Merck, 60–200 μm), which was used after heating for 18 h at 160°. Carazolol and β-D-glucuronidase were supplied by Boehringer Mannheim.

(R,S)-*3*-N-tert-*Butoxycarbonylisopropylamino-1-(carbazol-4-yloxy)-2-propa-*

nol (**2**) *and* (R,S)-*3*-N-tert-*butoxycarbonylisopropylamino-2*-tert-*butoxycarbonyloxy-1-(carbazol-4-yloxy)propane* (**3**). — Di-*tert*-butyl carbonate (4.80 g, 22.0 mmol) was added to a solution of **1** (3.04 g, 10.2 mmol) in 1,4-dioxane (40 mL) and 0.5M sodium hydroxide (20 mL) cooled in ice. The suspension was stirred for 30 min at room temperature and monitored by t.l.c. (hexane–ether, 1:1) After removal of the 1,4-dioxane, the aqueous solution was covered with ethyl acetate, cooled, acidified with dilute aqueous $KHSO_4$ to pH 2.5, and extracted twice with ethyl acetate. The combined extracts were washed thrice with water, dried (Na_2SO_4), and concentrated. The crude products were eluted from a column of silica gel (70 g) with dichloromethane–ether (20:1).

Eluted first was **2** (3.18 g, 78%), m.p. 104–106° (from toluene). ^{1}H-N.m.r. data (270 MHz, $CDCl_3$): δ 8.29 (d, 1 H, $J_{5',6'}$ 7.8 Hz, H-5'), 7.39–7.36 (m, 2 H, H-6',7'), 7.30 (m, 1 H, H-2'), 7.21 (m, 1 H, H-8'), 7.03 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1'), 6.66 (d, 1 H, $J_{2',3'}$ 8.0 Hz, H-3'), 4.37–4.16 (m, 4 H, H-1,1,2 and C*H*Me_2), 3.57 (m, 2 H, H-3,3), 1.50 (s, 9 H, CMe_3), 1.21 (d, 3 H, *J* 6.9 Hz, CH*Me*), 1.14 (d, 3 H, *J* 6.8 Hz, CH*Me*).

Anal. Calc. for $C_{23}H_{30}N_2O_4$ (398.4): C, 69.35; H, 7.54; N, 7.04. Found: C, 69.09; H, 7.47; N, 6.91.

Eluted second was **3** (415 mg, 12%). ^{1}H-n.m.r. data ($CDCl_3$): δ 8.33 (d, 1 H, $J_{5',6'}$ 7.7 Hz, H-5'), 7.41–7.16 (m, 4 H, H-2',6',7',8'), 7.05 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 6.61 (d, 1 H, $J_{2',3'}$ 7.9 Hz, H-3'), 5.51 (m, 1 H, H-2), 4.42–4.27 (m, 3 H, H-1,1 and C*H*Me_2), 3.81–3.56 (m, 1 H, H-3a), 3.43 (m, 1 H, H-3b), 1.50, 1.48 (2 s, 18 H, 2 CMe_3), 1.24 (d, 3 H, *J* 6.9 Hz, CH*Me*), 1.19 (d, 3 H, *J* 7.0 Hz, CH*Me*).

Anal. Calc. for $C_{28}H_{38}N_2O_6$ (498.4): C, 67.50; H, 7.63; N, 5.62. Found: C, 67.12; H, 7.81; N, 5.48.

Methyl [R-(+)- (**4**) *and* S-(−)-*3*-N-tert-*butoxycarbonylisopropylamino-1-(carbazol-4-yloxy)prop-2-yl 2,3,4-tri*-O-*acetyl-β*-D-*glucopyranosid]uronate* (**5**). — To a stirred suspension of **2** (1.2 g, 3.0 mmol), molecular sieve (4Å, 2.5 g), and Ag_2CO_3 (2.3 g, 8.3 mmol) in toluene (50 mL) was added, during 2 h, a solution of methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl bromide)uronate (3.0 g, 7.6 mmol) in toluene (20 mL). The mixture was stored for 96 h at room temperature (t.l.c.; chloroform–ethyl acetate, 4:1) under nitrogen with the exclusion of moisture and light, then diluted with ethyl acetate, filtered, and concentrated *in vacuo*. The crude product was eluted from columns of silica gel (30 and 70 g) with chloroform–ethyl acetate (20:1) and ether–hexane (4:1) to give an oily mixture (1.73 g, 82%) of **4** and **5**.

Anal. Calc. for $C_{36}H_{46}N_2O_{13}$ (714.1): C, 60.50; H, 6.44; N, 3.92. Found: C, 60.83; H, 6.57; N, 4.12.

Methyl [R-(+)- (**6**) *and* S-(−)-*1-(carbazol-4-yloxy)-3-isopropylaminoprop-2-yl 2,3,4-tri*-O-*acetyl-β*-D-*glucopyranosid]uronate* (**7**). — To a solution of the mixture (900 mg, 1.3 mmol) of **4** and **5** in dichloromethane (15 mL) and carbon tetrachloride (3 mL) was added trifluoroacetic acid (3 mL). After 15 min (t.l.c.; dichloromethane–methanol, 20:1), the solution was concentrated in a stream of

nitrogen. The crude products were eluted from a column of silica gel (60 g) with dichloromethane–methanol (24:1).

Eluted first was **6** (354 mg, 46%), $[\alpha]_D$ −1° (*c* 1.3, chloroform). ^{1}H-N.m.r. data (270 MHz, $CDCl_3$): δ 8.23 (d, 1 H, $J_{5',6'}$ 7.7 Hz, H-5′), 7.39–7.20 (m, 4 H, H-2′,6′,7′,8′), 7.01 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 6.58 (d, 1 H, $J_{2',3'}$ 7.9 Hz, H-3′), 5.24 (dd, 1 H, $J_{2'',3''}$ 9.1, $J_{3'',4''}$ 9.2 Hz, H-3″), 5.16 (dd, 1 H, $J_{4'',5''}$ 9.4 Hz, H-4″), 5.07 (dd, 1 H, $J_{1'',2''}$ 7.8 Hz, H-2″), 4.89 (d, 1 H, H-1″), 4.49 (dd, 1 H, $J_{1a,1b}$ 9.7, $J_{1a,2}$ 4.8 Hz, H-1a), 4.36 (m, 1 H, H-2), 4.13 (dd, 1 H, $J_{1b,2}$ 6.2 Hz, H-1b), 3.86 (d, 1 H, H-5″), 3.52 (s, 3 H, COOMe), 3.10 (dd, 1 H, $J_{3a,3b}$ 12.4, $J_{2,3a}$ 3.3 Hz, H-3a), 2.89 (dd, 1 H, $J_{2,3b}$ 8.6 Hz, H-3b), 2.81 (q, 1 H, *J* 6.2 Hz, C*H*Me$_2$), 2.10, 2.01, 1.95 (3 s, 9 H, 3 OAc), 1.09 (d, 6 H, 2 CH*Me*$_2$).

Anal. Calc. for $C_{31}H_{38}N_2O_{11}$ (614.1): C, 60.59; H, 6.19; N, 4.56. Found: C, 60.12; H, 6.03; N, 4.21.

Eluted second was **7** (398 mg, 51%), $[\alpha]_D$ −5.6° (*c* 1.6, chloroform). ^{1}H-N.m.r. data (270 MHz, $CDCl_3$): δ 8.19 (dd, 1 H, $J_{5',6'}$ 7.6, $J_{5',7'}$ 1.1 Hz, H-5′), 7.44 (dd, 1 H, $J_{7',8'}$ 7.9, $J_{6',8'}$ 1.4 Hz, H-8′), 7.38 (ddd, 1 H, $J_{6',7'}$ 6.9 Hz, H-7′), 7.28 (ddd, 1 H, H-6′), 7.25 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 7.9 Hz, H-2′), 7.11 (d, 1 H, H-1′), 6.44 (d, 1 H, H-3′), 5.21 (dd, 1 H, $J_{2'',3''}$ 9.3 Hz, $J_{3'',4''}$ 9.0 Hz, H-3″), 5.13 (dd, 1 H, $J_{4'',5''}$ 9.5 Hz, H-4″), 4.99 (dd, 1 H, $J_{1'',2''}$ 7.9 Hz, H-2″), 4.66 (d, 1 H, H-1″), 4.19 (m, 1 H, H-2), 4.05 (d, 1 H, H-5″), 4.03 (dd, 1 H, $J_{1a,1b}$ 9.9, $J_{1a,2}$ 6.4 Hz, H-1a), 3.86 (dd, 1 H, $J_{1b,2}$ 3.5 Hz, H-1b), 3.76 (s, 3 H, COOMe), 3.14 (dd, 1 H, $J_{3a,3b}$ 12.6, $J_{2,3a}$ 2.1 Hz, H-3a), 3.06 (q, 1 H, *J* 6.4 Hz, C*H*Me$_2$), 2.86 (dd, 1 H, $J_{2,3b}$ 9.0 Hz, H-3b), 2.04, 1.96, 1.48 (3 s, 9 H, 3 OAc), 1.25, 1.23 (2 d, 6 H, 2 CH*Me*$_2$).

Anal. Found: C, 60.73; H, 6.00; N, 4.38.

R-(+)- (**8**) *and* S-(−)-*1-(carbazol-4-yloxy)-3-isopropylaminoprop-2-yl* β-D-*glucopyranosiduronic acid* (**9**). — Compounds **6** and **7** (250 mg, 0.41 mmol) were each treated with methanolic 0.05M sodium methoxide (30 mL) for 12 h at room temperature (t.l.c.; methanol–water, 4:1). After acidification with methanolic 0.1M hydrochloric acid to pH 4.0, each solution was concentrated *in vacuo*, and the residue was eluted from a column of silica gel (30 g) with methanol. Aqueous solutions of **8** and **9** were lyophilised to give white amorphous powders.

Compound **8** (178 mg, 92%) had $[\alpha]_D$ −4.7° (*c* 0.9, methanol). ^{1}H-N.m.r. data (400 MHz, D_2O, 50°): δ 7.94 (d, 1 H, $J_{5',6'}$ 7.8 Hz, H-5′), 7.37–7.07 (m, 4 H, H-2′,6′,7′,8′), 6.97 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 6.45 (d, 1 H, $J_{2',3'}$ 8.0 Hz, H-3′), 4.53 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.22–4.15 (m, 2 H, H-1,1), 4.07–3.98 (m, 1 H, H-2), 3.77 (d, 1 H, $J_{4'',5''}$ 9.7 Hz, H-5″), 3.64 (dd, 1 H, $J_{3'',4''}$ 9.0 Hz, H-4″), 3.58 (dd, 1 H, $J_{2'',3''}$ 8.8 Hz, H-3″), 3.49 (dd, 1 H, H-2″), 2.98–2.81 (m, 3 H, H-3,3 and C*H*Me$_2$), 1.07 (d, 3 H, *J* 6.9 Hz, CH*Me*), 1.02 (d, 3 H, *J* 6.8 Hz, CH*Me*).

Anal. Calc. for $C_{24}H_{30}N_2O_8$ (474.2): C, 60.76; H, 6.33; N, 5.91. Found: C, 60.43; H, 6.18; N, 6.07.

Compound **9** (180 mg, 93%) had $[\alpha]_D$ −18.5° (*c* 0.9, methanol). ^{1}H-N.m.r. data: δ 8.10 (d, 1 H, $J_{5',6'}$ 7.6 Hz, H-5′), 7.57–7.14 (m, 5 H, H-1′,2′,6′,7′,8′), 6.63 (d, 1 H, $J_{2',3'}$ 7.8 Hz, H-3′), 4.57 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.40–4.30 (m, 1 H,

H-2), 4.24–4.11 (m, 2 H, H-1,1), 3.74 (d, 1 H, $J_{4'',5''}$ 9.5 Hz, H-5''), 3.64 (dd, 1 H, $J_{3'',4''}$ 8.9 Hz, H-4''), 3.53 (dd, 1 H, $J_{2'',3''}$ 8.9 Hz, H-3''), 3.46 (dd, 1 H, H-2''), 3.31–3.07 (m, 3 H, H-3,3 and C*H*Me_2), 1.28 (d, 3 H, *J* 6.8 Hz, CH*Me*), 1.25 (d, 3 H, *J* 6.6 Hz, CH*Me*).

Anal. Found: C, 60.92; H, 6.11; N, 5.83.

Mass spectra for the trimethylsilylated derivatives: **8**, *m/z* 762 (M^+) 747, 675, 579, 508, 464, 375, 300, 255, 222, 183, 147, 72; **9**, *m/z* 762 (M^+), 747, 690, 579, 523, 464, 375, 309, 255, 222, 183, 147, 72.

Hydrolysis with β-D-glucuronidase. — A solution (2 mL) of β-D-glucuronidase (from *E. coli*, 200 U/mL) at 37° was added to separate solutions (30 mg in 2 mL of 0.1M phosphate buffer at pH 6.3) of **8** and **9**, and the mixtures were incubated for 72 h at 37° and then treated with M sodium hydroxide (1 mL). Each mixture was extracted twice with ether, and the material extracted was purified by elution with methanolic 0.1% ammonia from columns of silica gel (30 g); $[\alpha]_D$ values (*c* 1, acetic acid) for the Carazolol obtained were +18.2° for the *R*-(+) form from **8** and −17.6° for the *S*-(−) form from **9**; lit.[13] −18.8° for the *S*-(−) form.

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