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Oxidation of β-blocking agents — VII. Periodate oxidation of labetalol*

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Abstract: The oxidation of labetalol with sodium metaperiodate is described. In spite of the bulky substituent on the amino group of labetalol, glycol cleavage of the molecule occurred. Spectrometric methods verified that the aromatic aldehyde formed was 2-hydroxy-5-formylbenzamide and that the amine was 1-methyl-3-phenylpropylamine. The polarographic behaviour of the aldehyde was examined as well. The half-wave potential in Britton–Robinson buffer pH 5.68 was −1.24 V. Direct linearity (r = 0.9999) was observed between the diffusion current and the concentration of the aldehyde. Quantitation of labetalol was carried out polarographically by measuring the concentration of 2-hydroxy-5-formylbenzamide formed in the oxidation.

Keywords: *Labetalol; β-blocker; periodate; oxidation; polarography.*

Introduction

Labetalol (**1**, Scheme 1) (2-hydroxy-5[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino] ethyl]benzamide) is an adrenergic β-receptor blocking agent used in the treatment of hypertension. Pharmacologically, **1** differs from the other β-blockers by also blocking α-receptors. Chemically, it is an arylethanolamine, in which the usual substituent of the terminal amino function of β-blockers, isopropyl or t-butyl, is replaced by the bulkier 1-methyl-3-phenylpropyl group. Periodate oxidation of β-blockers containing the isopropyl s- or t-butyl group in the amino function has been reported earlier [2]. The products of the oxidation were arylaldehyde or aryloxyacetaldehyde, formaldehyde, isopropyl or s- or t-butylamine [2].

In the present study, the effect of the bulky amino substituent is examined on the periodate oxidation of **1**. The oxidation products were isolated and their structures verified. Advantage was taken of the polarographic activity of one of the products, which was used for the polarographic assay of **1**.

Experimental

Chemicals and apparatus

Labetalol hydrochloride was kindly supplied by Leiras Pharmaceutical Company

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(Finland). The identity and purity of the substance was verified by thin-layer chromatography (TLC) and by UV, IR, and ^{1}H and ^{13}C NMR spectra. All other reagents and solvents were of analytical grade.

Elemental analyses were performed by Ilse Beetz Microanalytical Laboratory (Kronach, FRG). Melting points were determined with an Electrothermal Digital Melting Point apparatus and are uncorrected. The UV spectra were recorded using a Varian Techtron Model 635 spectrometer and the IR spectra using a Unicam SP3-200 instrument. The 200 MHz ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JMN-FX 200FT spectrometer using TMS as internal standard. The GC–MS spectra were recorded with a quadrupole mass spectrometer (HP 5970A) (electron energy 70 eV) coupled to an HP 5890 gas chromatograph. The analyses were carried out on a silica capillary column (BP-1) using a temperature programme 100–230°C/10°C min^{-1}. The temperature of both the injector and the detector was 240°C. TLC experiments were carried out on pre-coated 0.25 mm silica-gel aluminium 60 F_{254} plates. The solvent systems were: *n*-propanol–1% ammonia (4:1, v/v; A); methanol–1% ammonia (4:1, v/v; B); acetone–methanol (4:1, v/v; C); dichloromethane–methanol–25% ammonia (85:14:1, v/v/v; D). The spots were detected under UV light (254 nm) and by spraying with 2,4-dinitrophenylhydrazine (2,4-DNPH) in 2 M HCl or with ninhydrin reagent. The DC polarographic measurements were carried out on a polarograph E354/E261 (Metrohm) with Ag/AgCl reference electrode under the following conditions: starting voltage, 0 V; voltage range −2 V; sensitivity, 5×10^{-9} A mm^{-1}; damping, 2; compensating current, 25.

Oxidation of **1**

The hydrochloride salt of **1** (0.15 mmol) was dissolved in 10 ml of water. Thereafter, 0.5 ml of saturated sodium bicarbonate solution and 2.5 ml of 0.3 M sodium meta-periodate were added and the mixture was shaken for 10 min at room temperature. The products were isolated by two different methods: (1) The oxidation solution was extracted with methyl isobutyl ketone, the extract evaporated to dryness and the residue crystallized from water (compound **2**); (2) The oxidation solution was extracted with dichloromethane and the extract was evaporated to dryness. The residue was dissolved in a mixture of *n*-propanol–1% ammonia (4:1, v/v) for the flash chromatographic [3] purification. The same solvent system was used as eluent. The combined fractions of the desired component were evaporated to dryness and the purity of the oily residue (compound **3**) was checked by TLC. The flash chromatographic purification was carried out twice.

Polarography

The dependence of the half-wave potential and the wave height of **2** (0.04 mM solution) on pH was investigated in Britton–Robinson buffers from 2.67 to 8.97.

Calibration curve. Solutions for the calibration curve were prepared from a methanolic stock solution of **2** (2 mM), by diluting it with Britton–Robinson buffer solution pH 5.68 to obtain aldehyde concentrations of 0.02–0.1 mM.

Assay of the pure substance. About 50 mg of **1** as the hydrochloride salt was oxidized by the method described above under "Oxidation of **1**". The oxidation was stopped by treating the mixture with 30 ml of methanol and 3 ml of 10% barium chloride solution.

The suspension was allowed to stand for 30 min, with shaking from time to time, and then was filtered through a sintered glass filter (3 G 4). The filtrate was diluted to 100.0 ml by washing the flask and residue with methanol. An aliquot of 2 ml of this solution was diluted to exactly 50 ml with buffer solution and polarographed under the conditions mentioned above.

Assay of tables (Albetol® 100 mg). The determinations were carried out on single tablets. Each tablet was allowed to disintegrate in 20 ml of distilled water, subsequently stirred for 10 min (magnetic stirrer). The suspensions were treated as described above under "Assay of the pure substance".

Oxidation products

2-Hydroxy-5-formylbenzamide (**2**). White crystals from water. M_r 165.15, m.p. 204–206°C. Found: C, 58.09; H, 4.24; N, 8.83; O, 28.74. Calc. for $C_8H_7NO_3$: C, 58.18; H, 4.27; N, 8.48; O, 29.06. UV λ_{max} in methanol: 297 (ϵ = 8500) nm. IR ν_{max} (KBr disc): 3440–3220 (phenol —OH and primary amide —$CONH_2$), 3080(aromatic —CH), 1690(—CHO), 1660(amide **I**), 1615(amide **II**), 1590 and 1485(aromatic ring), 1445, 1370, 1275, 1195, 945, 910, 825, 730, 650, 610 cm^{-1}. ^1H NMR [DMSO-d_6] δ: 14.04(s, 1H, OH), 9.89(s, 1H, CHO), 8.57(d, 1H, aromatic), 8.50(d, 2H, NH_2), 8.02–7.96(m, 1H, aromatic), 7.11(d, 1H, aromatic) ppm. ^{13}C NMR [DMSO-d_6] δ: 190.5(d, CHO), 171.3(s, $CONH_2$), 166.3(s, aromatic C_2), 134.6(d, aromatic C_4), 131.6(d, aromatic C_6), 127.8(s, aromatic C_5), 118.4(d, aromatic C_3), 114.8(s, aromatic C_1) ppm. MS *m/z* (% rel.int.): 165.2(M, 91.8), 148.1(100.0), 119.1(56.6), 92.1(24.4), 79.1(6.5), 63.1(25.1), 53.0(19.7), 44.0(19.0).

1-Methyl-3-phenylpropylamine (**3**). Colourless, oily product. M_r 149.23, m.p. of benzoyl derivative 107–108°C [4]. Found: C, 80.53; H, 7.40; N, 5.57; O, 6.34. Calc. for $C_{11}H_{19}NO$ (benzoyl derivative): C, 80.60; H, 7.56; N, 5.53; O, 6.32. UV λ_{max} in methanol: 252(ϵ = 160), 308(ϵ = 70) nm. IR ν_{max} (KBr disc): 3370(NH_2), 3070 and 3040(aromatic —CH), 2980–2860(aliphatic —CH), 1610 and 1590(aromatic ring and NH_2), 1500 and 1460(aromatic ring), 1380, 1070, 1030, 840, 750, 700 cm^{-1}. ^1H NMR [$CDCl_3$] δ: 7.31–7.16(m, 5H, aromatic), 2.99–2.89(m, 1H, CH), 2.71–2.61(m, 2H, CH_2), 2.24(s, 2H, NH_2), 1.73–1.62(m, 2H, CH_2), 1.13(d, 3H, CH_3) ppm. ^{13}C NMR [$CDCl_3$] δ: 142.1(s, aromatic, C_1), 128.4(d, aromatic, $C_{2,3,5,6}$), 125.8(d, aromatic, C_4), 46.7(d, CH), 41.4(t, CH_2), 32.8(t, CH_2), 23.6(q, CH_3) ppm. MS *m/z* (% rel.int.): 149.2(M, 0.5), 132.2(9.8), 117.1(6.9), 91.1(15.3), 77.1(3.9), 65.1(5.4), 44.1(100.0).

Results and Discussion

Compound **1** was oxidized with periodate as easily as the β-blockers studied earlier. According to the TLC experiments, an oxidation time for 10 min at room temperature was sufficient for the complete cleavage of the molecule. Two major oxidation products, tentatively designated compounds **2** and **3**, were observed on the TLC plates: 2,4-DNPH caused **2** to go yellow, and ninhydrin caused **3** to go purple, indicating carbonyl and amine character, respectively. The R_f values in different eluents are summarized in Table 1.

Structure elucidation of the products

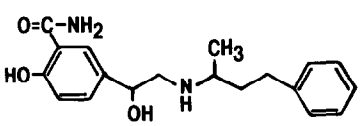
Compound **2**. The characteristic features of the IR spectrum of **2** were the absorptions

Table 1
The results of the TLC experiments

	R_f values in the eluent system			
Compound	A	B	C	D
1	0.70	0.80	0.20	0.45
2	0.80	0.90	0.80	0.10
3	0.35	0.30	0.65	0.80

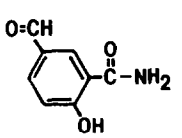
belonging to the carbonyl and primary amide groups. The mass spectrum showed the molecular ion peak at *m/z* 165.2, corresponding to the formula $C_8N_7NO_3$. Elemental analysis gave the same result which was also supported by the ^1H and ^{13}C NMR spectra. In the ^1H NMR spectrum the carbonyl group in the *o*-position shifted the phenolic proton absorption downfield (δ14.04 ppm) because of intramolecular hydrogen bonding. The two-proton doublet at δ8.50 ppm was due to the amino group of primary amide. After the addition of a drop of D_2O the absorptions caused by phenolic proton and amide protons disappeared. The one-proton singlet at δ9.89 ppm indicated that the carbonyl group belonged to an aldehyde. The downfield shifts in the ^{13}C NMR spectrum were due to the carbons of the aldehyde and primary amide groups, and the phenolic OH caused a downfield shift in the signal for C_2. The chemical shifts were consistent with those calculated for 2-hydroxy-5-formylbenzamide, and since all other data supported the same conclusion, compound **2** was positively identified as 2-hydroxy-5-formylbenzamide (Scheme 1).

Compound **3**. Absorptions due to the primary amine function were clearly apparent in the IR spectrum. The molecular ion peak was observed at *m/z* 149.2 in the mass spectrum, corresponding to the formula $C_{10}H_{15}N$. The elemental analysis of the benzoyl derivative of **3** confirmed the mass spectrometric result. The integral of the ^1H NMR spectrum indicated a total of 15 protons in **3**. Five of the protons belonged to the aromatic ring, and the molecule contained in addition one methyl and one methine group and two methylene groups. The primary amine protons were responsible for the broad signal at δ2.24 ppm. The structure of **3** was confirmed by the ^{13}C NMR spectrum as 1-methyl-3-phenylpropylamine (Scheme 1). Formaldehyde was identified as a third oxidation product, by the method described earlier [1].

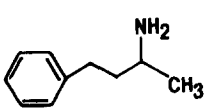


1

Scheme 1



2



3

Polarography

Compound **2** contains the polarographically active carbonyl group, which allows it to be reduced on the dropping mercury electrode. Like aromatic aldehydes generally, **2** was reduced in the very negative region and both the half-wave potential and the wave height were dependent on pH (Fig. 1). The polarographic reduction of **2** provided a means for the determination of **1**. Before measurement, periodate and iodate were eliminated from the oxidation solution with barium chloride [5]. In the Britton–Robinson buffer (pH 5.68) used for the quantitative measurements, the half-wave potential of **2** was −1.24 V. The correlation coefficient of 0.9999 showed direct linearity between the diffusion current and concentration in the range 3.0–18.0 μg ml^{-1}. The calibration curve could be described by the equation: $y = 2.8429x - 0.7667$. The results of the quantification of **1** by polarographic determination of **2** are comparable with the results obtained by perchloric acid titration of **1** (Table 2). The accuracy is good enough for the determination of **1** in pharmaceutical preparations, and the precision is satisfactory.

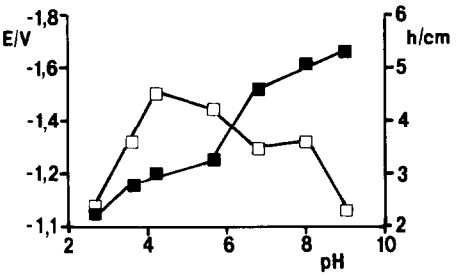


Figure 1
The dependence of the half-wave potential (■) and the wave height (□) of **2** on pH.

Table 2
Comparison of the results obtained from polarography and 0.1 M perchloric acid titration of **1**

	Polarography		Titration	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Pure substance (n = 10)	98.9	3.06	101.4	1.28
Tablets 100 mg (n = 10)	102.4	3.53	102.2	2.69

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