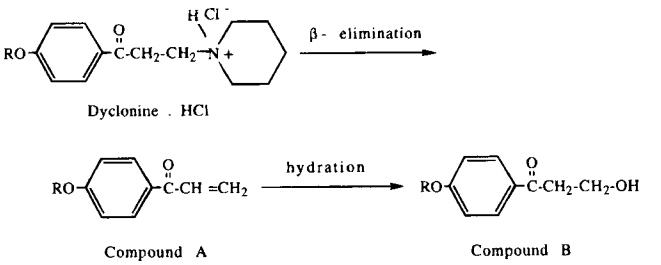
Isolation and Characterization of Two Major Degradation Products of Dyclonine Hydrochloride

CHENG-YI LIANG*, Y. YANG*, MOHAMMAD A. KHADIM‡, GILBERT S. BANKER*, AND VIJAY KUMAR*x

Received January 9, 1995, from the *Division of Pharmaceutics, College of Pharmacy, University of Iowa, Iowa City, IA 52242 and ‡Hoechst Celanese Corporation, 4331 Chesapeake Drive, Charlotte, NC, 28216-3410. Accepted for publication June 14, 1995⊗.

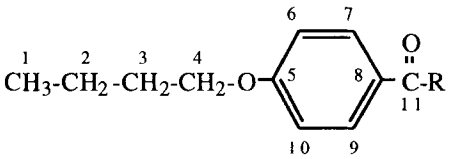
Abstract □ Dyclonine hydrochloride, a local anesthetic, is known to degrade in aqueous media. In this paper, the isolation and characterization of two major degradation products, formed by heating of an aqueous solution of dyclonine hydrochloride for 2 weeks at 50 °C, are presented. The proton and carbon-13 nuclear magnetic resonance, infrared, and mass spectral data reported conclusively show the two products to be 1-(4-butoxyphenyl)-2-propen-1-one and 1-(4-butoxyphenyl)-3-hydroxy-1-propanone. The proton and carbon-13 nuclear magnetic resonance spectral data of the free dyclonine base are also included.



Scheme 1—degradation pathways of dyclonine hydrochloride (R = $CH_3CH_2CH_2CH_2$).

Dyclonine hydrochloride, 1-(4-butoxyphenyl)-3-(1-piperidinyl)-1-propanone hydrochloride (Scheme 1), is a local anesthetic used to anesthetize mucous membranes prior to endoscopy, suppress the gag reflex, relieve the pain due to minor burns and local sore throat, alleviate the discomfort of gynecologic or proctologic procedures, and manage pruritus ani or vulvae.[1] It is known to degrade in water. The degradation rate is 47.5 times faster at pH 4.5 than at pH

Table 1—^{1}H NMR Spectral Assignment of the Isolated Products

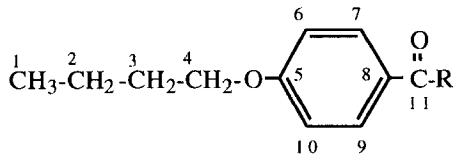


Free Dyclonine Base[a]		Compound A[b]		Compound B[a]	
R = -CH2-CH2-N (12 13; 14 15; 16; 17 18)		R = H, H, C=C, H (12; 13a; 13b)		R = -CH2-CH2OH (12 13 14)	
Chemical shift (δ, ppm)	Multiplicity[c], number of H, assignment, coupling constant (*J*, Hz)	Chemical shift (δ, ppm)	Multiplicity[c], number of H, assignment, coupling constant (*J*, Hz)	Chemical shift (δ, ppm)	Multiplicity[c], number of H, assignment, coupling constant (*J*, Hz)
7.94	d, 2H, $H_{7,9}$, J = 8.9	7.99	d, 2H, $H_{7,9}$, J = 10.8	7.93	d, 2H, $H_{7,9}$, J = 8.6
6.91	d, 2H, $H_{6,10}$, J = 8.8	7.32	dd, 1H, H_{12}, J = 10.8; 18.0	6.92	d, 2H, $H_{6,10}$, J = 8.6
4.01	t, 2H, H_4, J = 6.4	7.02	d, 2H, $H_{6,10}$, J = 10.8	4.03	m, 4H, $H_{4,13}$
3.17	t, 2H, H_{12}, J = 7.1	6.37	dd, 2H, H_{13b}, J = 3.6; 18.0	3.17	t, 2H, H_{12}, J = 5.2
2.81	t, 2H, H_{13}, J = 7.9	5.89	dd, 1H, H_{13a}, J = 3.6; 10.8	3.01	broad, 1H, H_{14}
2.48	m, 4H, $H_{14,18}$	4.06	t, 2H, H_4, J = 3.6	1.78	m, 2H, H_3
1.77	m, 2H, H_3	1.78	m, 2H, H_3	1.51	m, 2H, H_2
1.54	m, 8H, $H_{2,15,16,17}$	1.52	m, 2H, H_2	0.98	t, 3H, H_1, J = 7.3
0.98	t, 3H, H_1, J = 7.3	0.99	t, 3H, H_1, J = 7.2		

[a] In $CDCl_3$. [b] In CD_3OD. [c] Center of peaks: d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet.

0022-3549/95/3184-1141$09.00/0

Table 2—^{13}C NMR Spectral Assignment of the Isolated Products



Free Dyclonine Base[a]		Compound A[b]		Compound B[a]	
R = -CH_2-CH_2-N (ring)		R = -CH = CH_2		R = -CH_2-CH_2OH	
Chemical shift (δ, ppm)	Assignment	Chemical shift (δ, ppm)	Assignment	Chemical shift (δ, ppm)	Assignment
197.59	C_{11}	190.97	C_{11}	198.93	C_{11}
162.94	C_5	165.00	C_5	163.30	C_5
130.15	$C_{7,9}$	133.39	$C_{7,9}$	130.25	$C_{7,9}$
129.56	C_8	132.23	C_{12}	129.40	C_8
113.98	$C_{6,10}$	130.95	C_8	114.14	$C_{6,10}$
67.73	C_4	129.73	C_{13}	67.85	C_4
54.41	$C_{14,18}$	115.48	$C_{6,10}$	58.12	C_{13}
53.92	C_{13}	69.10	C_4	39.80	C_{12}
35.68	C_{12}	32.32	C_3	30.96	C_3
30.68	C_3	20.25	C_2	19.05	C_2
25. 69	$C_{15,17}$	14.15	C_1	13.70	C_1
24.05	C_{16}				
19.04	C_2				
13.68	C_1				

[a] In $CDCl_3$. [b] In CD_3OD.

2.9 and increases by 3.7-fold with every 10 °C increase in temperature.[2] Between pH 1.0 and 2.2, dyclonine hydrochloride is stable.[2] The degradation mixture has been shown by high performance liquid chromatography to contain several degradation products.[2] Simat[3] isolated and investigated the structures of these products using infrared (IR) and nuclear magnetic resonance spectroscopy (NMR), but failed to provide any clues as to their identity other than that the products possess a 4-butoxyphenone moiety.

In this paper, we report the isolation and characterization of two major degradation products of dyclonine hydrochloride.

Experimental Section

Dyclonine hydrochloride was received from Rugar Chemicals (Rugar Chemical Co., Inc., Hillside, NJ). ^1H and ^{13}C NMR spectra were recorded on Varian AC-300, Bruker 250 MHz, and WM-360 spectrometers. Tetramethylsilane was used as an internal reference. IR spectra were recorded on a Nicolet 5DXB Fourier-transform infrared spectrophotometer. The electron-impact mass spectra (EI-MS) were obtained on a Ribermag R10–10C spectrometer using a solid probe. The gas chromatograph–mass spectrometer (GC–MS) system was a Hewlett-Packard 5890 gas chromatograph interfaced with a 5970 Selective Mass Detector. Samples were dissolved in chloroform and 1 μL was injected into the GC port. A DB-5 30 meter long capillary column was used with an initial temperature of 70 °C, programmed at 10 °C/min to 270 °C, and held for 20 min. Chromatographic separations were performed using Analtech 150A (35–75 μm) silica gel or preparative thin layer chromatography plates using Analtech 200 mm silica gel. All solvents used were either analytical or chromatographic grade.

Degradation of Dyclonine Hydrochloride—One liter of 0.5% (w/v) aqueous dyclonine hydrochloride solution was placed in a round-bottomed flask equipped with a condenser and was heated at 50 °C for 2 weeks. The solution became cloudy and developed a yellowish tint after 2 days of heating. Continued heating produced a clear solution with separation of fine yellow oil droplets. The pH of the solution dropped from an initial value of 4.8 to about 3.

Isolation of the Degradation Products—The degraded solution of dyclonine hydrochloride was cooled to room temperature and extracted with 6 × 200 mL of diethyl ether. The ethereal layer was separated, dried over anhydrous sodium sulfate, and concentrated under vacuum. The resulting semisolid residue (yield 0.14 g) was chromatographed by preparative TLC (ethyl acetate/hexane 1:1). Two products, compound **A** (R_f = 0.76) and compound **B** (R_f = 0.39), were isolated and characterized. Other degradation products were present in small amounts, and no further attempt was made to isolate them.

The aqueous layer, left after ether extraction, was treated with a saturated solution of sodium bicarbonate at room temperature. Extraction with diethyl ether produced an oil (yield 0.38 g), which was purified by column chromatography (ethyl acetate/hexane/triethylamine 1:2.0:0.1; R_f = 0.28) and characterized as the free dyclonine base (Tables 1 and 2).

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1995.

Results and Discussion

Compounds **A** and **B** and the free dyclonine base were characterized by IR, ^1H and ^{13}C NMR, and MS. The proton signals were assigned on the basis of distinct chemical shifts and coupling constants, whereas the assignment of the carbon-13 peaks was made from estimation of the substitution effects and using the DEPT (distortionless enhancement by polarization transfer) pulse sequence technique. DEPT-135 afforded methylene carbon signals with a phase opposite to those of the methine and methyl signals. Quaternary carbon signals did not appear in the spectrum.

The ^1H and ^{13}C NMR chemical shifts and their assignments are presented in Tables 1 and 2.

Compound A—Compound **A** is an α,β-unsaturated product, (1-(4-butoxyphenyl)-2-propen-1-one). It can be formed by a β-elimination reaction from the dyclonine hydrochloride (Scheme 1). The ^1H NMR spectrum of **A** shows a characteristic double doublet signal at δ 7.32 ppm due to the proton (H_{12}) located on the β-ethylene carbon (C_{12}). The two protons (H_{13a} and H_{13b}) at α-ethylene carbon (C_{13}) appear at δ 6.37 and 5.89 ppm as double doublets. From the peak pattern and the coupling constants, the signals at δ 6.37 and 5.89 ppm are assigned to *trans*- and *cis*-protons, with respect to the proton located on the β-carbon (C_{12}). A proton 2D homonuclear correlation (COSY) experiment clearly demonstrated correlations between each doublet of doublets located at δ 7.32, 6.37, and 5.89 ppm. The same experiment yielded correlations among butoxy group protons as well as among the aryl protons. The ^{13}C spectrum of **A** shows characteristic peaks at δ 190.97 and 165.0 ppm due to the >C=O and the phenolic C—O carbons, respectively. The upfield shift of the carbonyl carbon signal in **A**, compared to that in dyclonine base, is consistent with the presence of a conjugated carbonyl group.

The ν(C=O) stretching vibration in the IR spectrum of **A** appears at 1665 cm^{-1}, about 10 cm^{-1} lower in frequency from the position observed in free dyclonine base. This is attributed to the conjugation of the carbonyl group with an aryl group and an α,β-double bond.[4]

Compound **A**, under the GC—MS conditions used, elutes at 13.8 min. The mass spectrum shows a molecular ion peak at *m/e* 204 (65%) and a base peak at *m/e* 121 (100%) due to the $O{=}C_6H_4{=}C{=}O^+H \leftrightarrow HOC_6H_4C{\equiv}O^+$ fragment. The base peak results from $C_4H_9OC_6H_4C{\equiv}O^+$ (*m/e* 177; 15%) and $HOC_6H_4C(O)CH{=}CH_2$ (*m/e* 148), which, in turn, are formed from the parent ion by elimination of $C_2H_3^\bullet$ and C_4H_8 moieties. Other peaks in the spectrum appear at *m/e* 93 (30%) and 65 (40%) due to $C_6H_5O^+$ and $C_5H_5^+$ ions, forming from the fragment appearing at *m/e* 121 by successive elimination of CO.

Compound B—The formation of **B**, 1-(4-butoxyphenyl)-3-hydroxy-1-propanone, probably occurs by hydration of the α,β-unsaturated product **A**. The same mechanism has been proposed in the conversion of ethacrynic acid to the corresponding hydroxy derivative.[5,6] The mass spectrum of **B** did not show the molecular ion peak, but instead produced a peak at *m/e* 204, suggesting that **B** readily dehydrates and produces the α,β-unsaturated product **A** under the mass spectrum measurement conditions. The structure of **B**, however, was confirmed by ^1H and ^{13}C NMR (Tables 1 and 2). The peak at δ 3.01 ppm exchanged with D_2O, and thus, was assigned to the hydroxyl proton.

The ν(C=O) stretching frequency in the IR spectrum of **B** occurs at 1675 cm^{-1}. The corresponding peak in the free dyclonine base appears at 1678 cm^{-1}, suggesting that the carbonyl carbon in **B** has an electronic environment very similar to that in the free base.

References and Notes

1. *AMA Drug Evaluations*, 5th ed.; American Medical Association: Chicago, IL, 1983; p 386.
2. Bhagat, H. R.; Bhargava, H. N.; Williams, D. A. *J. Pharm. Biomed. Anal.* **1989**, *7*, 441–46.
3. Simat, M. M. S. Thesis, Massachusetts College of Pharmacy, 1984.
4. Pavia, D. L.; Lampman, G. M.; Kriz, G. S. *Introduction to Spectroscopy: A Guide for Students in Organic Chemistry*; Saunders College Publishing: Philadelphia, 1979.
5. Gupta, V. D. *Drug Dev. Ind. Pharm.* **1982**, *8*, 869–882.
6. Yarwood, R. J.; Moore, W. D.; Collett, J. H. *J. Pharm. Sci.* **1985**, *74*, 220–223.

JS950012W