

# Isolation and Identification of Three Potential Impurities of Pholcodine Bulk Drug Substance

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## Abstract

Three previously unreported manufacturing impurities were isolated from a pholcodine mother liquor using preparative reversed-phase HPLC.

The liquor was the residue remaining after recrystallisation of a production batch of pholcodine. The impurities, which are structurally related to pholcodine, were initially detected by thin-layer chromatography (TLC). Their structures were determined after separation by preparative HPLC (Econo-Prep 5  $\mu$ m C18 column, 30 cm  $\times$  21.2 mm i.d.). Structure elucidation was carried out using nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and ultra violet (UV) spectroscopy.

The impurities were identified as alkylated derivatives of pholcodine possessing second 2-morpholinoethyl substituents at various positions.

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Pholcodine [(5*R*,6*S*)-4,5-epoxy-*N*-methyl-3-(2-morpholinoethoxy)morphin-7-en-6-ol] (Figure 1) is an opiate derivative which is widely used as an anti-tussive agent and is available as an over-the-counter medicine in many countries. It is a semi-synthetic alkaloid, which was first synthesised in 1950 by Chabrier et al. In the original manufacturing process, a solution of morphine in water was treated with an equivalent amount of sodium hydroxide and then mixed with a solution of chloroethyl morpholine hydrochloride, previously neutralised with sodium hydroxide. The precipitated pholcodine was isolated by filtration and recrystallised from benzene. Stern (1955) improved the procedure by using alcohol as solvent and sodium ethoxide as base in the reaction mixture. In the work-up procedure, the sodium chloride precipitate was filtered off and the alcohol was removed by distillation. The residue was dissolved in water and made alkaline with sodium hydroxide, causing the precipitation of pholcodine. Further quantities of pholcodine could be obtained by extraction of the aqueous mother liquor with chloroform as outlined in the patent for morphine- $\beta$ -morpholinoether (1958).

Opium alkaloids are derived from the latex of the seed capsule of the opium poppy *Papaver somniferum* L. There is a great potential for the presence of impurities in the purified alkaloids, since plant materials are imported from various parts of the world and the content of alkaloids might vary considerably with origin (Remberg et al 1994). Since pholcodine is a semi-synthetic drug, impurities in pholcodine may arise from impurities present in the morphine starting material or from the manufacturing process itself. Because the morphine impurities are controlled at a low level, as described in the British Pharmacopoeia (BP) monograph (1998), then only morphine itself remains as a potential starting material impurity for which a limit may be set by using a colorimetric test (BP monograph, Pholcodine 1998).

Potential manufacturing impurities may arise from side reactions of chloroethylmorpholine with other sites in the morphine molecule. In this respect pholcodine would be analogous to other morphine ether derivatives such as codeine, where alkylation occurs on the allylic alcohol at the 6-position to give the major impurity (Ayyangar et al 1990).

In this study, the liquor remaining after the recrystallisation of pholcodine was examined. This liquor was used as source material for isolating potential impurities present in pholcodine bulk drug

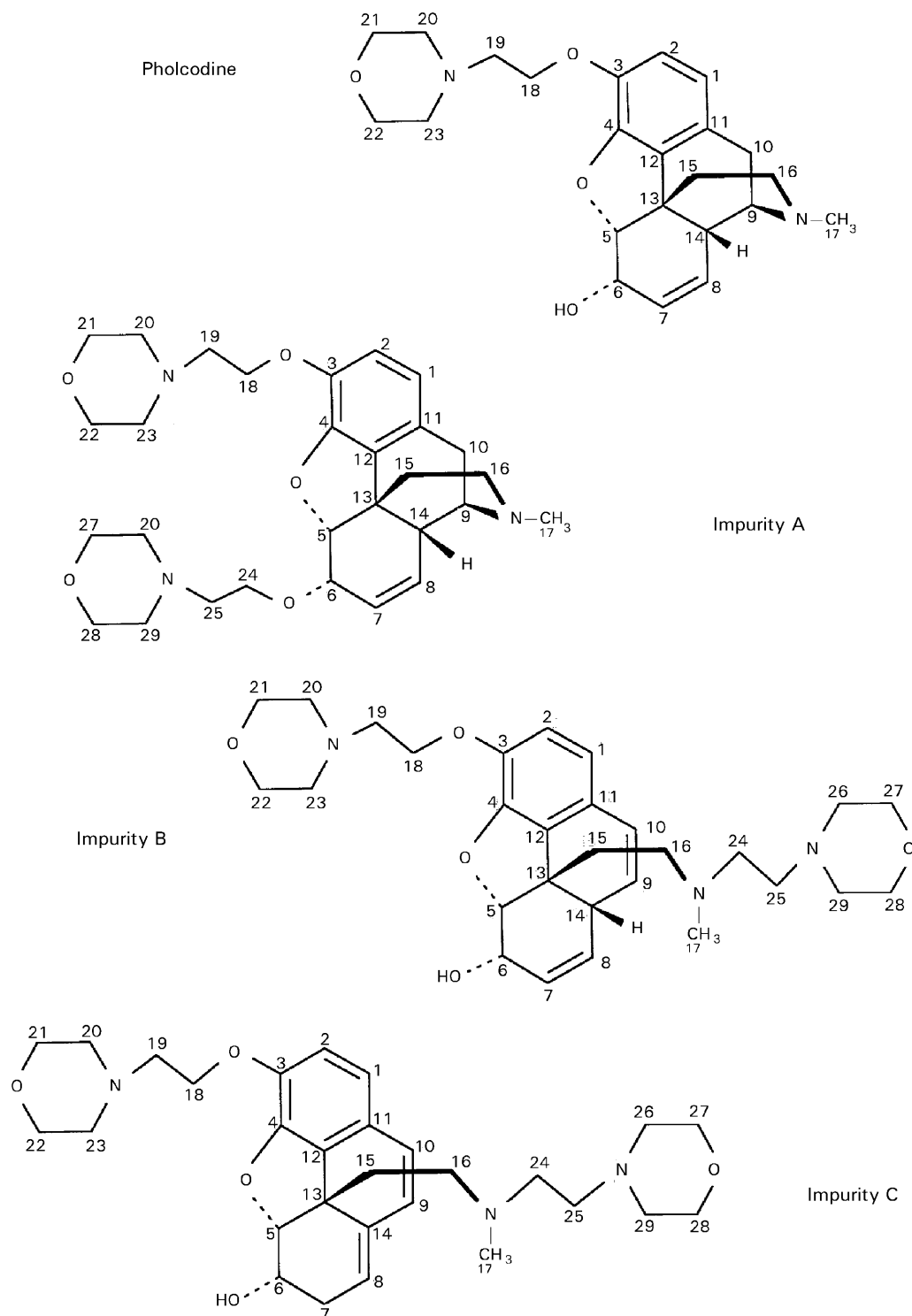


Figure 1. Structural formulae of pholcodine and the isolated impurities (A, B and C). Numbering scheme after Grewe (1947).

substance. In the course of our research we were able to isolate and identify three new compounds, which are derivatives of pholcodine. This paper describes the detection, isolation and structure elucidation of these new compounds.

## Materials and Methods

### Materials

A sample of an aqueous liquor from the purification of a production batch of pholcodine was supplied

by the manufacturer, Macfarlan Smith Ltd (Edinburgh, UK). A portion (200 mL) of this solution was extracted with three volumes ( $3 \times 200$  mL) of chloroform. The chloroform extracts were combined and evaporated to dryness with a rotary evaporator under reduced pressure to give the source material, hereafter referred to as chloroform extract.

The Dragendorff TLC visualising reagent used differed from the procedure described by Moffat et al (1986) and was prepared as follows: bismuth subnitrate (0.85 g) was dissolved in 50 mL of a solution of 20% (v/v) glacial acetic acid in water (Solution 1) and Solution 2 was potassium iodide (8 g) dissolved in 20 mL of water. A stock solution of the reagent was prepared by mixing 20 mL of each of the solutions. The reagent was stored for a maximum of two weeks at 4°C. The spray solution for the visualisation of the thin-layer chromatograms, was freshly prepared every day by mixing 4 mL of the stock solution with 4 mL of glacial acetic acid and 20 mL of water.

Pholcodine BP, morphine hydrochloride, codeine hydrochloride, chloroethyl morpholine (CEM) hydrochloride were supplied by Macfarlan Smith Ltd (Edinburgh, UK). Water was glass distilled and all other chemicals were of analytical grade or better. All HPLC solvents were filtered and degassed using a 0.45- $\mu$ m nylon filter (Whatman, Kent, UK).

#### *Thin-layer chromatography (TLC)*

TLC was performed on  $20 \times 20$  cm silica gel 60 F<sub>254</sub> polyester sheets obtained from E. Merck (Darmstadt, Germany). Portions (10  $\mu$ L) of standard solutions of each analyte (1% w/v in chloroform) were applied to the plates using capillary tubes. The TLC tank was lined with filter paper and the mobile phase (methanol/chloroform/13.5 M ammonium hydroxide; 70:30:1 by volume) was allowed to equilibrate for 30 min. Before application of the sample solutions, the commercial TLC plates were pre-developed in the mobile phase. The plates were then dried for 1 h at 60°C before application of the sample solutions. The TLC plates were developed until the solvent had travelled a distance of approximately 15 cm. The chromatograms were dried in the fume cupboard, before viewing under UV light at 254 nm and subsequent spraying with Dragendorff reagent.

The purified impurities obtained after fractionation with HPLC were analysed by TLC using one-dimensional double development. The samples were treated as described above. The TLC plate was developed a second time until the solvent

had travelled a distance of 17.5 cm. The plate was then allowed to dry in the fume cupboard, viewed under UV light (254 nm), and then sprayed with Dragendorff reagent.

#### *Preparative, gradient HPLC*

Isolation of the impurities was carried out in two steps. The first step was a crude fractionation of the extract mainly to remove the large amount of pholcodine. Thereafter, each fraction was further purified using two different HPLC systems depending on the analyte of interest.

All separations were carried out by gradient HPLC using two Gilson pumps (Villiers-le-Bel, France; Gilson-305 (master pump)/Gilson-307 (slave pump)) fitted with a mano-metric module (Gilson 806) and a 23-mL dynamic mixer (Gilson 811C). The system was equipped with a Rheodyne Model 7125 manual injector fitted with a 2-mL loop. The eluent was monitored at 220 nm using a Gilson 118 UV/VIS detector and data were collected using a Hewlett Packard HP3395 integrator (Waldbronn, Germany).

*HPLC System 1 (HS 1).* For the initial fractionation, a 50-mg-mL<sup>-1</sup> solution of the chloroform extract was prepared in mobile phase (20% v/v acetonitrile (ACN)/ammonium acetate buffer pH 6.8). Volumes ( $10 \times 2$  mL) of this solution were injected onto a Phenomenex (Macclesfield, UK) Econo-Prep 5  $\mu$ m C18 column (30 cm  $\times$  21.2 mm i.d.) fitted with a Phenomenex Econo-Prep C18 guard column (60  $\times$  21.2 mm i.d.). The initial mobile phase was a mixture of 15% (v/v) ACN/0.01 M ammonium acetate buffer adjusted to pH 6.8 with 1.35 M ammonium hydroxide. The linear gradient used in the separation, expressed as a percentage (v/v) of organic solvent B (ACN), was 15% isocratic until 5 min after the injection, which was increased to 25% at 10 min, and maintained for a further 25 min before finally increasing B to 80% at 50 min. The flow rate was 16 mL min<sup>-1</sup>. Six fractions (Figure 2) were collected manually, the mobile phase evaporated to dryness under reduced pressure and the residues dissolved in chloroform (4 mL). The chloroform solutions were quantitatively transferred into tared vials and chloroform was removed at 60°C under a stream of nitrogen. Fractions 5 and 6 were separately further purified using the same experimental conditions as were used for the initial separation.

*HPLC System 2 (HS 2).* Fraction 4 was further purified using a Hypersil (Shandon, Runcorn, UK) BDS 5  $\mu$ m C18 column (25 cm  $\times$  21.2 mm i.d.) with

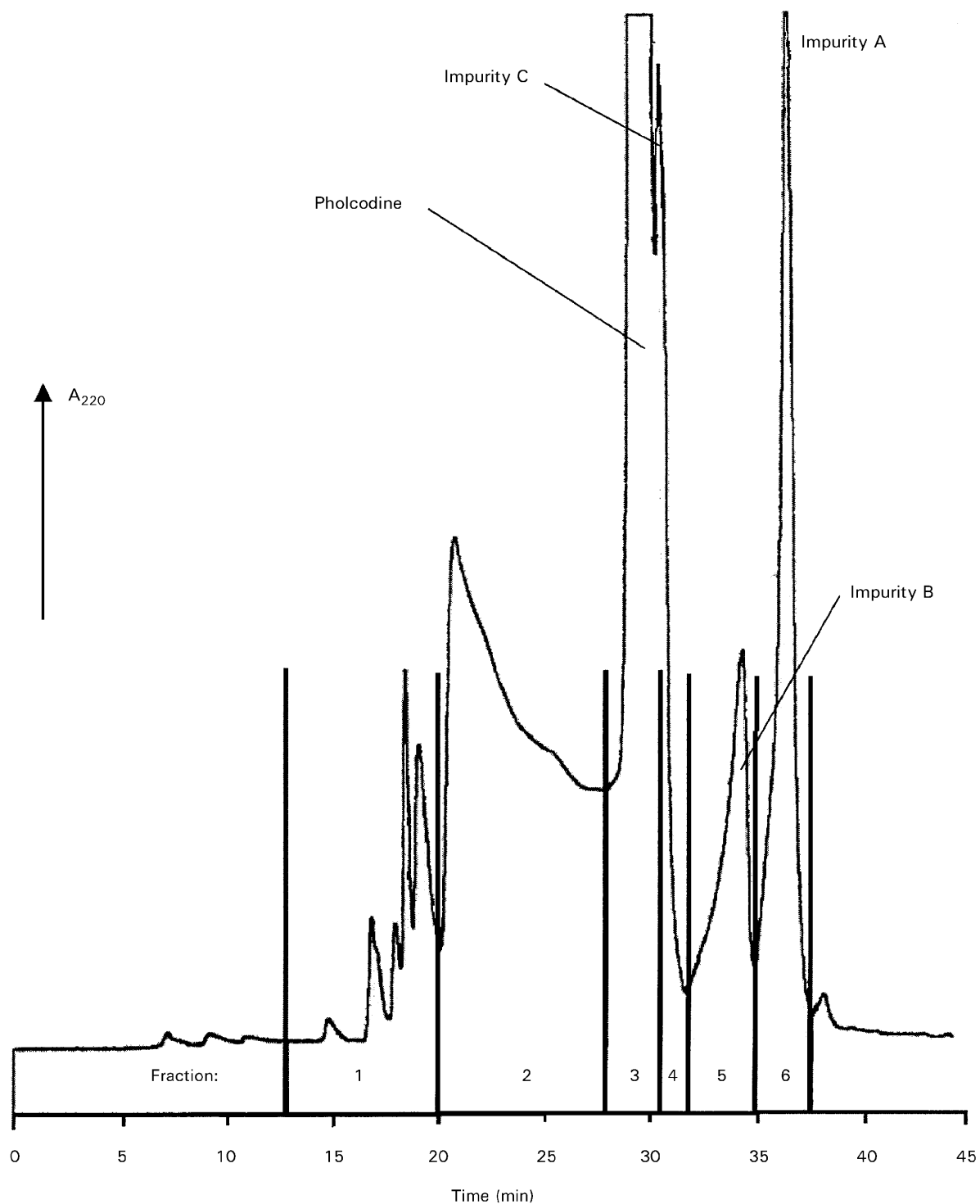


Figure 2. HPLC chromatogram of a  $50\text{-mg}\cdot\text{mL}^{-1}$  solution of the chloroform extract of pholcodine mother liquor injected onto a Phenomenex Econo-Prep  $5\text{-}\mu\text{m}$  C18 column ( $30\text{ cm} \times 21.2\text{ mm i.d.}$ ). The mobile phase was a mixture of acetonitrile and  $0.01\text{ M}$  ammonium acetate buffer adjusted to  $\text{pH } 6.8$ . A two-step linear gradient was used with a flow rate of  $16\text{ mL min}^{-1}$  (HPLC System 1, HS1).

a Phenomenex Econo-Prep C18 guard column ( $60 \times 21.2\text{ mm i.d.}$ ). The initial mobile phase consisted of a mixture of  $15\%$  (v/v) ACN/ammonium acetate buffer adjusted to  $\text{pH } 8.0$  with  $1.35\text{ M}$  ammonium hydroxide. The linear gradient used in

the separation, expressed as the percentage (v/v) of organic solvent B (ACN), was an increase from  $15\%$  to  $17.5\%$  of B until  $10\text{ min}$  after the injection of sample. The mobile phase composition was then maintained at  $17.5\%$  B for  $40\text{ min}$ , followed by an

increase to 40% B for 30 min, which was then left to run isocratically for 10 min before re-equilibration of the column. The flow rate was 16 mL min<sup>-1</sup>.

Each fraction was manually collected and the mobile phase was removed under reduced pressure. The residues from each fraction were dissolved in approx. 50 mL of chloroform, washed once with 25 mL of a saturated solution of sodium hydrogen carbonate and dried over anhydrous sodium sulphate. The sodium sulphate was filtered off and the chloroform was removed under reduced pressure. The residues in the flasks were again dissolved in chloroform, transferred into storage vials and the chloroform was evaporated at 60°C under a stream of nitrogen.

**Nuclear magnetic resonance spectroscopy (NMR)**  
<sup>1</sup>H and <sup>13</sup>C NMR spectra, two-dimensional homopolar correlation spectra (<sup>1</sup>H-<sup>1</sup>H-COSY), heteronuclear multiple quantum coherence (HMQC) spectra and heteronuclear multiple bond correlation (HMBC) spectra were recorded for the isolated impurities and for pholcodine. The samples were prepared for analysis by dissolving approximately 15 mg of each compound in 1 mL of deuterated chloroform. Signals were referenced to the solvent chloroform at  $\delta_{\text{H}}$  7.27 and  $\delta_{\text{C}}$  77.23. Spectra were recorded at 400.13 MHz <sup>1</sup>H and 100.61 MHz <sup>13</sup>C on a Bruker DPX400 NMR spectrometer. The atomic numbering scheme used was that of Grewe (1947) for unsubstituted morphine extended to include the morpholinoethyl substitution at various positions (Figure 1).

#### Mass spectrometry

Mass spectrometry was performed on each compound using a Jeol AX505 instrument. The samples were directly inserted into the mass spectrometer. The mode of ionisation was electron impact (EI) at 70 eV for pholcodine and the three impurities. Elemental analyses of three impurities were performed on the same instrument using the high-resolution mode. High-resolution spectra were calibrated against perfluorokerosin (PFK).

**Impurity A:** calculated for C<sub>29</sub>H<sub>41</sub>N<sub>3</sub>O<sub>5</sub>: 511.3046; found: 511.3078 ( $\Delta$  -6.2 ppm). **Impurity B:** calculated for C<sub>29</sub>H<sub>41</sub>N<sub>3</sub>O<sub>5</sub>: 511.3046; found: 511.3025 ( $\Delta$  4.2 ppm). **Impurity C:** calculated for C<sub>29</sub>H<sub>41</sub>N<sub>3</sub>O<sub>5</sub>: 511.3046; found: 511.3097 ( $\Delta$  -9.9 ppm).

#### Ultra-violet (UV) spectrometry

The UV spectra were recorded using an Unicam V1.30 UV-Visible spectrometer. Aqueous solutions

of each compound (approx. 1 mg mL<sup>-1</sup>) were measured against water using a bandwidth of 2.0 nm and a data interval of 1.0 nm.

## Results and Discussion

#### TLC analysis of the chloroform extract

An aqueous mother liquor obtained from the pholcodine production process was thought to be the best source material for potential impurities of pholcodine present in bulk drug substance. Preliminary TLC analysis of this liquor showed that its composition was very complex with up to 15–20 different compounds being detected by visualisation with UV light and Dragendorff reagent. Thus, chloroform extraction of the aqueous mother liquor was carried out since impurities likely to be present in pholcodine bulk drug substance should be co-extractable with chloroform. The chloroform extract was analysed by TLC and compared with pure standards of Pholcodine BP, codeine HCl, morphine HCl and CEM HCl. Apart from the principal pholcodine spot ( $R_{\text{f}}$ =0.36) two extra spots, one above ( $R_{\text{f}}$ =0.43) and one below ( $R_{\text{f}}$ =0.24) the pholcodine spot were detected in the chloroform extract (Table 1).

#### Gradient HPLC isolation of impurities A–C from the chloroform extract

During method development it was soon discovered that the chloroform extract appeared to contain three major impurities and at least five minor compounds (Figure 2). Two types of LC stationary phases were examined for their suitability to separate the main three analytes of interest, but unfortunately baseline separation for

Table 1. Summary of  $R_{\text{f}}$  values obtained after TLC analysis of chloroform extract of mother liquor from pholcodine production.

Sample	$R_{\text{f}}$ value	UV <sub>254</sub>	Dragendorff
CEM HCl	0.70	–	Violet
Codeine HCl	0.42	+	Orange
Morphine HCl	0.37	+	Orange
Pholcodine	0.36	+	Intense orange
CHCl <sub>3</sub> extract	0.65	–	Violet
	0.42	+	Orange
	0.36	+	Intense orange
	0.23	+	Orange
	0.12	–	Faint orange

Mobile phase: methanol/chloroform/13.5 M ammonium hydroxide, (70/30/1 by volume). Stationary phase: silica gel 60 F<sub>254</sub> plastic sheets (20 × 20 cm).

the three major impurities could not be achieved. In the reversed-phase HPLC system using a 10- $\mu\text{m}$  Econo-prep C18 stationary phase (HS 1), impurities A and B were essentially baseline separated eluting at 36.7 and 34.6 min, respectively, but impurity C appeared as a shoulder on the pholcodine peak at 30.8 min in the chromatogram (Figure 2).

Although it was possible to achieve baseline separation of impurity C (65.0 min) from pholcodine using a base deactivated Hypersil stationary phase (HS 2), A and B co-eluted with each other at 68.7 min (Figure 3). It was confirmed that the peak in the chromatogram of fraction 2 (Figure 2) at 20.9 min obtained using HS 1 was due to pholcodine.

The isolation of the impurities was performed in two steps. Starting from the crude chloroform extract, 10 injections were made using HS 1. The amounts of material obtained for each fraction are given in Table 2. Impurities A and B were further purified using the same system (HS 1). Impurity C was further purified using HS 2. This second purification step yielded 57.0 mg of impurity A, 58.4 mg of impurity B and 12.5 mg of impurity C (Table 2).

#### TLC analysis of the isolated impurities

The isolated impurities were analysed by TLC as described in the experimental section and compared with pure standards of pholcodine and the chloroform extract. All three isolated compounds produced a single spot in the TLC chromatogram (Table 3). Impurities A and C had  $R_f$  values of 0.13 and 0.26, respectively. Impurity B co-migrated with pholcodine ( $R_f = 0.22$ ).

#### Structure elucidation

The structural identification of the impurities was accomplished by detailed NMR investigations. This required complete  $^1\text{H}$  and  $^{13}\text{C}$  assignments for pholcodine (Table 4). Some signal assignments were straightforward on the basis of their characteristic chemical shifts and multiplicities (e.g. the aromatic carbons C-1 and C-2 or the olefinic carbons C-7 and C-8). Other hydrogen-bearing carbon atoms and the corresponding protons were identified by tracing  $^{13}\text{C}$ - $^1\text{H}$  and  $^1\text{H}$ - $^1\text{H}$  coupling via HMQC and COSY experiments. The quaternary carbons were assigned unambiguously from 2D long-range correlation measurements (HMBC). Complete  $^1\text{H}$  and  $^{13}\text{C}$  signal assignments for pholcodine and the three impurities are detailed in Table 4.

Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of pholcodine and impurity A showed that all of the

pholcodine-type signals were present in the  $^1\text{H}$  spectrum of impurity A except for the hydroxyl group ( $\delta_{\text{H}}$  2.90). Additionally, the  $^1\text{H}$  and  $^{13}\text{C}$  spectra of impurity A had signals for a second morpholinoethoxy substituent. In the HMBC spectrum of impurity A correlations between the 18- $\text{CH}_2$  protons and C-3 and between 24- $\text{CH}_2$  protons and C-6 confirmed the attachment of the two morpholinoethoxy substituents in positions 3 and 6 suggesting that A is (5*R*, 6*S*)-3,6-di-(2-morpholinoethoxy)-4,5-epoxy-*N*-methylmorphin-7-en (Figure 1).

In pholcodine the two 18- $\text{CH}_2$  protons are non equivalent ( $\delta_{\text{H}}$  4.09 ddd;  $\delta_{\text{H}}$  4.23 ddd) but in the  $^1\text{H}$  spectrum of A, the 18- $\text{CH}_2$  protons were almost equivalent ( $\delta_{\text{H}}$  4.20 m). Furthermore, the 24- $\text{CH}_2$  protons in A are non-equivalent ( $\delta_{\text{H}}$  3.68 ddd,  $\delta_{\text{H}}$  3.90 ddd) similar to the 18- $\text{CH}_2$  of pholcodine (Table 4). These data indicate that, in the case of pholcodine, the 18- $\text{CH}_2$  protons prefer a conformation which places them in different environments. Probably due to steric hindrance the morpholinoethoxy substituent at position 3 of impurity A is forced out of this position resulting in two almost equivalent 18- $\text{CH}_2$  protons. Contrarily, the 24- $\text{CH}_2$  protons of the second morpholinoethoxy substituent of impurity A adopts a similar conformation as the 18- $\text{CH}_2$  protons in pholcodine, which results in two non-equivalent protons.

Analysis of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of impurity B again showed the presence of two morpholinoethoxy groups. The 18- $\text{CH}_2$  protons ( $\delta_{\text{H}}$  4.15 m and  $\delta_{\text{H}}$  4.28 m) were similar to those of pholcodine and correlated in the HMBC spectrum with C-3. The 24- $\text{CH}_2$  protons at  $\delta_{\text{H}}$  2.51 were more shielded than those in impurity A and correlated with C-16 (53.9 ppm). The chemical shift of the latter carbon is significantly different ( $\Delta = 7.4$  ppm) from that of pholcodine and impurity A (Table 4). The most dramatic differences can be seen in positions 9 and 10. For pholcodine and impurity A, the 9-CH resonates at ca.  $\delta_{\text{H}}$  4.4 (ca.  $\delta_{\text{C}}$  59) and the 10- $\text{CH}_2$  resonates at ca.  $\delta_{\text{H}}$  2.3/3.0 (ca.  $\delta_{\text{C}}$  20.8). In contrast, for impurity B, the 9-CH shifts to  $\delta_{\text{H}}$  6.0 ( $\delta_{\text{C}}$  129.5) and the 10-position converts to a CH resonating at  $\delta_{\text{H}}$  6.50 ( $\delta_{\text{C}}$  125.3) (Table 4), indicating the formation of a 9,10-double bond. These data indicate that impurity B has arisen through *N*-morpholinoethylation with formation of an N-C-24 bond and concomitant scission of the N-C-9 bond. Impurity B is therefore 3-hydroxy-4a-[*N*-methyl-*N*-(2-morpholinoethyl)-aminoethyl]-6-(2-morpholinoethoxy)-4,5-oxa-3,4,4a,10a-tetrahydrophenanthrene (Figure 1). The morphine numbering used for impurities B and C in Figure 1 does not comply with the IUPAC system, but allows a better

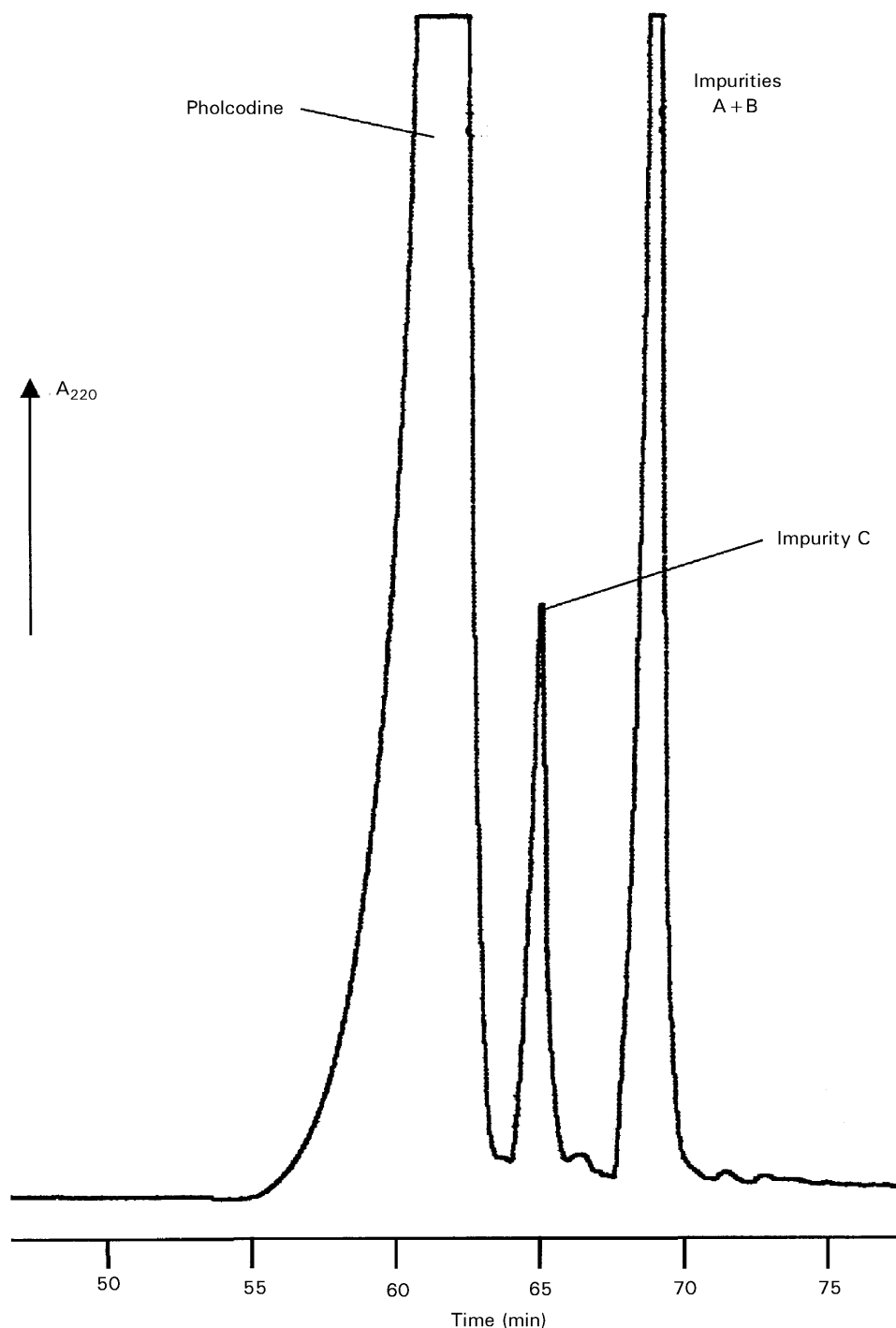


Figure 3. HPLC chromatogram of the chloroform extract solution injected onto a Hypersil BDS 5- $\mu$ m C18 column (25 cm  $\times$  21.2 mm i.d.). The mobile phase consisted of acetonitrile and 0.01 M ammonium acetate buffer (pH 8.0) delivered at a flow rate of 16 mL min<sup>-1</sup> using two-step linear gradient elution (HPLC System 2, HS2).

comparison of pholcodine with the isolated compounds.

<sup>1</sup>H and <sup>13</sup>C spectra of impurity C showed it to be the C-8, C-9 diene isomer of impurity B. The <sup>13</sup>C signal of C-7 has shifted from 132.2 ppm to 29 ppm (Table 4). The <sup>1</sup>H NMR data in conjunction with

COSY and HMQC experiments proved that C-7 possesses two protons with chemical shifts of  $\delta_{\text{H}}$  2.35 and  $\delta_{\text{H}}$  2.61. Furthermore, CH-9 appears as a doublet ( $\delta_{\text{H}}$  6.16) coupling only with H-10 while in impurity B the corresponding proton had an additional coupling with H-14. This conversion of C-14

Table 2. Yield and purities of the isolated pholcodine impurities.

Impurity	CHCl <sub>3</sub> extract (Area %) <sup>a</sup>	Crude Separation		Purification	
		Yield (mg)	Purity (w/w)	Yield (mg)	Purity (w/w)
A	20.2%	218.0	68%	57.0	>98%
B	10.2%	134.0	82%	58.4	>98%
C	3.7%	115.0	78%	12.5	>92%

<sup>a</sup>Peak area percentage values of the HPLC chromatogram shown in Figure 1. A similar detector response for the three impurities can be assumed from the UV data obtained.

from a CH ( $\delta_C$  37.8) to a quaternary carbon ( $\delta_C$  140.1) was confirmed by HMBC correlations. Thus it is concluded that impurity C is 3-hydroxy-4a-[N-methyl-N-(2-morpholinoethyl)-aminoethyl]-6-(2-morpholinoethoxy)-4,5-oxa-2,3,4,4a-tetrahydrophe-nanthrene (Figure 1).

Table 3. Summary of R<sub>f</sub> values obtained after TLC analysis of the isolated pholcodine impurities.

Sample	R <sub>f</sub> value <sup>a</sup>	UV <sub>254</sub>	Dragendorff
Pholcodine	0.22	+	Intense orange
CHCl <sub>3</sub> extract	0.13	+	Orange
	0.23	+	Orange
	0.24	+	Orange
	0.26	+	Faint orange
	0.13	+	Orange
Impurity A (fraction 6)	0.13	+	Orange
Impurity B (fraction 5)	0.22	+	Intense orange
Impurity C (fraction 4)	0.26	+	Faint orange
Fraction 2	0.23	+	Intense orange

<sup>a</sup>R<sub>f</sub> values given for second development. Mobile phase: methanol/chloroform/13.5 M ammonium hydroxide (70/30/1 by volume). Stationary phase: silica gel 60 F<sub>254</sub> plastic sheets (20 × 20 cm).

*Confirmation of structures by MS and UV analysis*  
Each compound was analysed by MS (Table 5). All three impurities produced the expected molecular ion of m/z 511. Fragmentation of the molecular

Table 4. NMR chemical shift data of pholcodine and impurities A, B and C.

C-No.	Pholcodine		Impurity A		Impurity B		Impurity C	
	<sup>1</sup> H NMR $\delta_H$ (ppm)	<sup>13</sup> C NMR $\delta_C$ (ppm)	<sup>1</sup> H NMR $\delta_H$ (ppm)	<sup>13</sup> C NMR $\delta_C$ (ppm)	<sup>1</sup> H NMR $\delta_H$ (ppm)	<sup>13</sup> C NMR $\delta_C$ (ppm)	<sup>1</sup> H NMR $\delta_H$ (ppm)	<sup>13</sup> C NMR $\delta_C$ (ppm)
1	6.51 (d)	119.7	6.49 (d)	119.2	6.54 (d)	118.2	6.64 (d)	118.8
2	6.63 (d)	115.7	6.65 (d)	116.5	6.67 (d)	115.2	6.65 (d)	115.1
3	–	141.0	–	141.2	–	142.8	–	142.6
4	–	147.1	–	148.1	–	146.8	–	145.7
5	4.84 (d)	91.7	4.96 (d)	89.7	5.19 (d)	90.5	5.02 (d)	90.5
6	4.14 (m)	66.6	3.92 (m)	74.9	4.23 (m)	66.2	4.33 (m)	67.1
7	5.25 (td)	128.5	5.30 (td)	128.5	5.29 (td)	127.8	2.35 (d)	29.0
							2.61 (t)	
8	5.65 (d)	133.5	5.71 (d)	131.2	5.81 (d)	132.2	5.72 (dd)	122.2
9	3.32 (m)	58.9	3.42 (m)	59.1	6.00 (dd)	129.5	6.16 (d)	125.3
10	3.01 (d)	20.6	3.04 (d)	20.9	6.50 (d)	125.3	6.39 (d)	124.8
	2.26 (dd)		2.36 (dd)					
11	–	128.0	–	127.4	–	124.4	–	124.4
12	–	131.6	–	131.1	–	129.4	–	131.2
13	–	43.0	–	43.0	–	45.4	–	45.1
14	2.64 (m)	40.9	2.73 (m)	40.8	2.87 (m)	37.8	–	140.1
15	2.03 (dt)	35.9	2.07 (dt)	35.8	1.93 (m)	32.6	1.84 (dt)	34.2
	1.84 (d)		1.89 (d)		2.15 (m)		1.98 (dt)	
16	2.37 (dt)	46.5	2.46 (dt)	46.6	2.61 (m)	53.9	2.60 (m)	53.5
	2.54 (t)		2.65 (m)		2.33 (m)		3.39 (m)	
17	2.40 (s)	43.2	2.47 (s)	43.3	2.29 (s)	42.6	2.30 (s)	42.5
18	4.23 (ddd)	67.2	4.20 (m)	67.6	4.28 (m)	67.2	4.30 (m)	67.0
	4.09 (ddd)				4.15 (m)		4.20 (m)	
19	2.72 (t)	57.9	2.76 (t)	58.6	2.77 (t)	57.8	2.80 (t)	57.8
20, 23	2.52 (m)	54.0	2.57 (m)	54.3	2.56 (m)	54.3	2.60 (m)	54.2
21, 22	3.71 (t)	66.8	3.73 (t)	67.1	3.74 (t)	67.0	3.74 (t)	67.1
24	–	–	3.90 (ddd)	67.0	2.51 (m)	56.4	2.50 (m)	56.3
			3.68 (ddd)					
25	–	–	2.67 (t)	58.1	2.53 (m)	56.5	2.52 (m)	56.3
26, 29	–	–	2.57 (m)	54.3	2.44 (m)	54.2	2.45 (m)	54.2
27,28	–	–	3.73 (t)	67.1	3.69 (t)	66.9	3.69 (t)	67.1
–OH	2.90 (s)	–	–	–	3.00 (s)	–	2.80 (s)	–

s = singlet, dd = doublet of doublet, ddd = doublet of doublet of doublet, dt = doublet of triplet, td = triplet of doublet, t = triplet and m = multiplet.



ions was consistent with loss of morpholinomethyl ring ( $m/z$  100) and morpholinoethyl ( $m/z$  114) radicals for all three compounds and pholcodine. The formation of an ion with an  $m/z$  value of 411 was characteristic for impurities B and C indicating loss of a morpholinomethyl radical to produce a stable fragment (Figure 4).

The UV spectra of pholcodine and impurities A, B and C were examined to obtain further information about the structures of these compounds. The UV spectrum of impurity A was found to be identical with that obtained for pholcodine (Table 5). This was not surprising, since the addition of the morpholinoethyl moiety at position 6

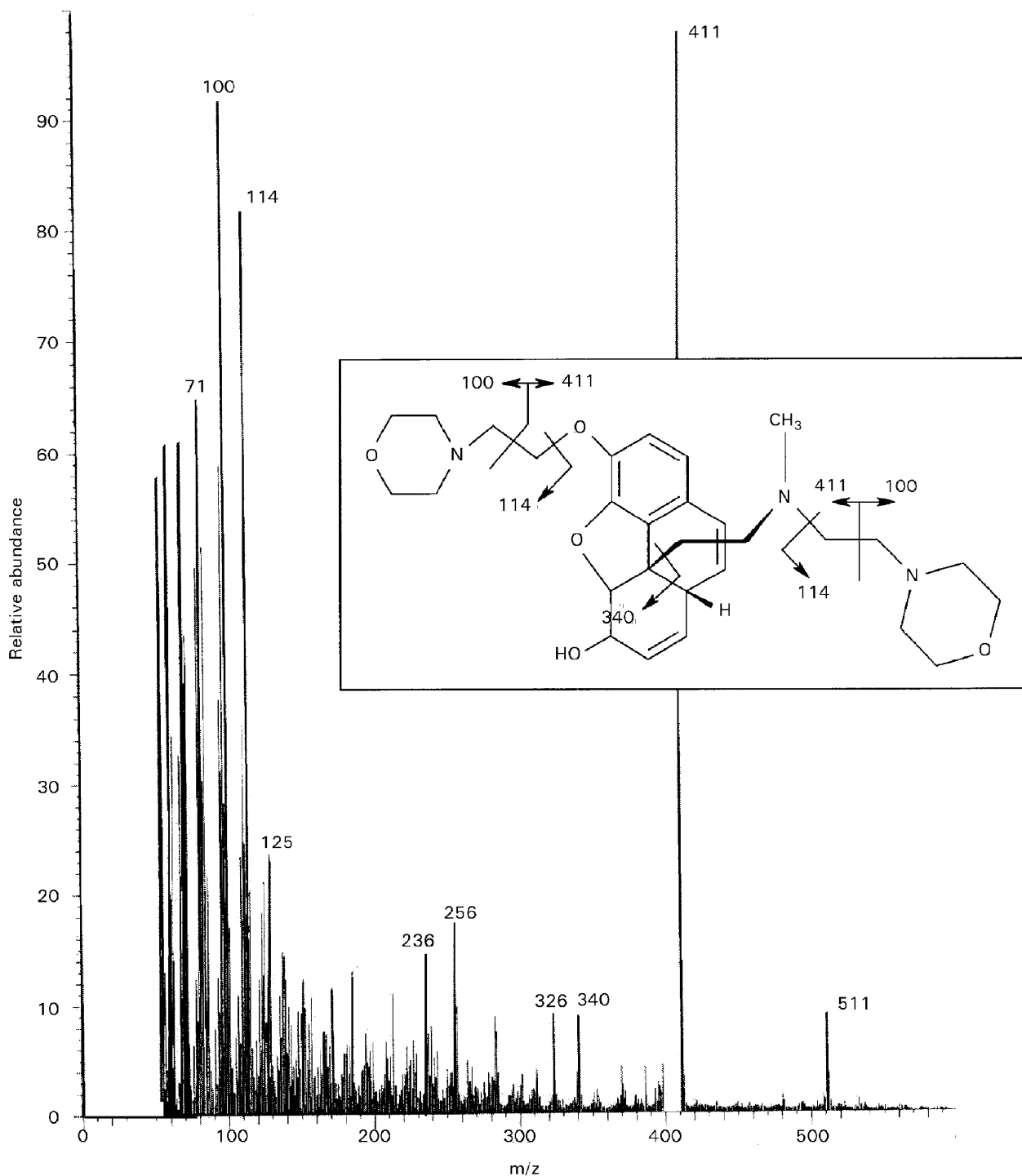


Figure 4. Mass spectrum of impurity B with proposed fragmentations.

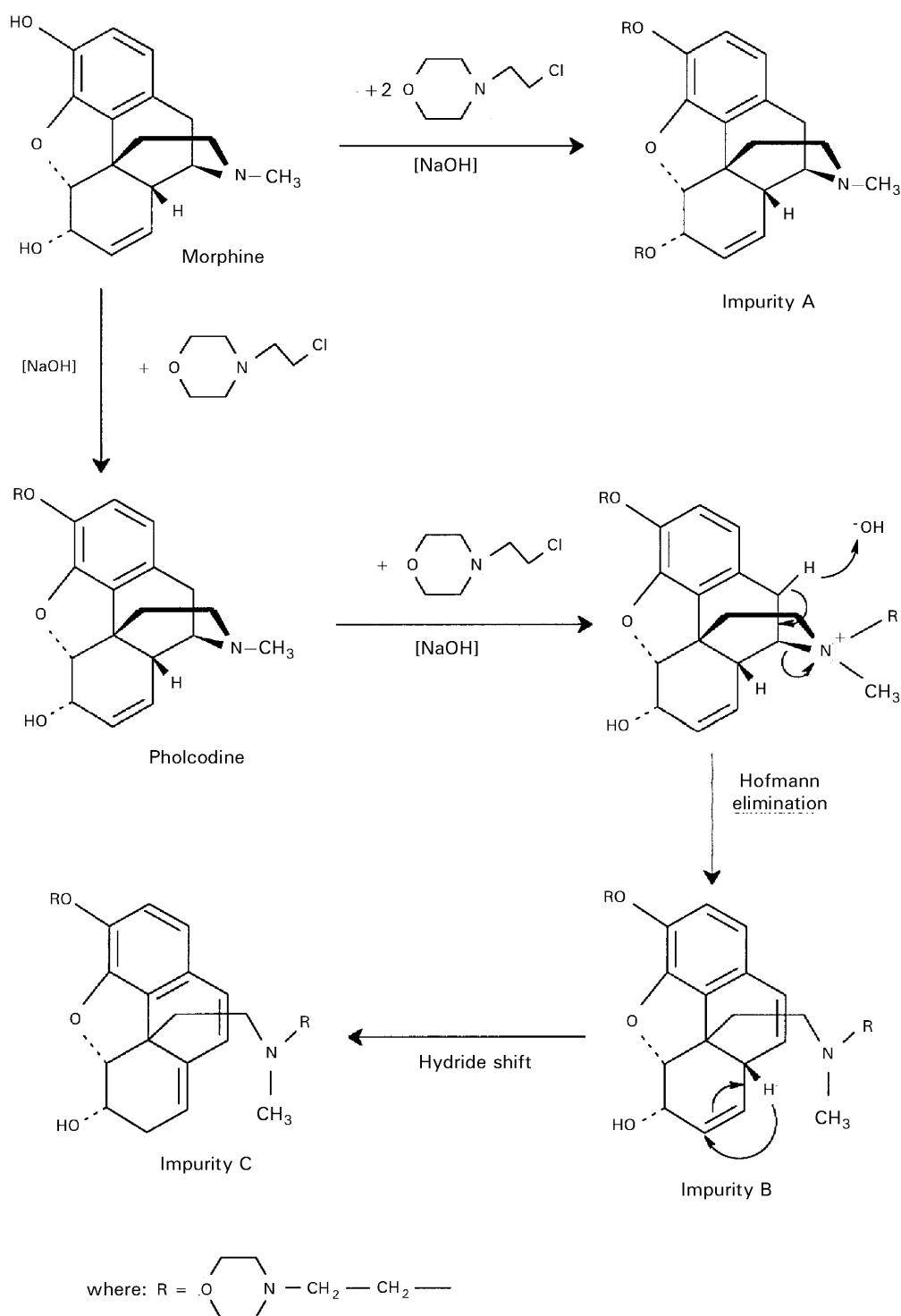


Figure 5. Proposed reaction pathway for the formation of the impurities.

had no influence on the chromophore. Impurity B possessed an additional double bond, which extended the conjugated system of the aromatic ring resulting in two UV maxima for this compound (Table 5). The shift of the allylic double

bond in case of impurity C led to the formation of a fully conjugated diene system with the aromatic ring, explaining its much longer wavelength absorption maxima when compared with impurity B (Table 5).

Table 5. Mass-spectral results and UV maxima of pholcodine and the isolated impurities.

Compound	Ions (m/z)			UV maxima (nm)	
	Molecular ion	Base peak	Fragments <sup>a</sup>	Primary	Secondary
Pholcodine	398	114	100, 56, 115, 101, 70, 284	284	–
Impurity A	511	114	100, 399, 56, 70, 368, 268	284	–
Impurity B	511	411	100, 114, 71, 341, 231, 169	229	270
Impurity C	511	411	100, 114, 70, 341, 171, 236	246	314

<sup>a</sup>In order of their abundance.

The formation of these three by-products by the alkylation of morphine with chloroethylmorpholine is fairly easy to understand, although it might be expected that the alkylation of the alcoholic OH group would require a stronger base than sodium ethoxide to give impurity A. Impurities B and C arise from the alkylation of the nitrogen atom. After quaternisation of the nitrogen atom, a subsequent Hofmann elimination (Cope & Trumbull 1960) occurs in the presence of base to form impurity B. Impurity B then undergoes an allylic shift to form impurity C, which has a fully conjugated system with the aromatic ring (Figure 5).

The presence of these impurities in samples of pholcodine is currently being investigated using micellar electrokinetic capillary chromatography (MEKC) and analytical HPLC and will be reported at a later stage.

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