Solid-State Esterification of Codeine Phosphate by the Acid Constituent of Effervescent Tablets

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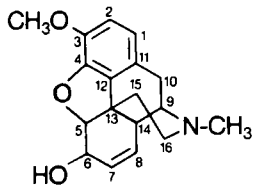
Abstract □ Codeine phosphate in a paracetamol:codeine effervescent tablet was found to react at room temperature and 37 °C with the citric acid constituent to form citrate esters of codeine. The esterification was confirmed in a solid-state reaction at elevated temperature. The structures of the three possible monosubstituted esters (**1–3**) were elucidated from spectroscopic data (nuclear magnetic resonance and mass spectrometry) and by selective hydrolysis of the dimethyl esters to give symmetrical and asymmetrical dimethyl citrates. In the degradation reaction, formation of the symmetrically substituted citrate ester of codeine, **1**, was found to predominate. Tartaric acid, which can be used in effervescent tablet formulations, was also found to give an ester with codeine phosphate in a similar nonsolvolytic reaction. A liquid chromatographic method was developed for the separation of the citrate esters of codeine.

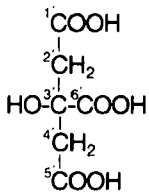
This work was initiated by the discovery of unknown degradation products during the development of a paracetamol:codeine effervescent tablet. Compatibility studies indicated interaction of codeine phosphate with the citric acid constituent. Such degradation was also observed when tartaric acid was used instead of citric acid. Although several effervescent tablet formulations containing codeine alone or in combination with other analgesics are marketed, there appears to be very little reported about possible interactions of codeine with the acid component of the formulation.

Only a few cases of solid-state ester formation have been described in the literature. The most well known is the acetylation of codeine (and some other substances) by aspirin by solid-state transesterification.[1,2] On the other hand, in liquid dosage forms, citric[3] and tartaric acids[4] have been shown to have a stabilizing effect on codeine.

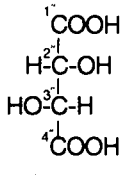
This report describes the solid-state synthesis, isolation, and structural elucidation of the esters of codeine and citric acid, and codeine and tartaric acid.



Codeine



Citric acid



Tartaric acid

Experimental Section

Reagents and Chemicals—Codeine phosphate hemihydrate (MacFarlan Smith, Lot 13955), citric acid (Benckiser, Lot 234283), and (2*R*, 3*R*)-2,3-dihydroxysuccinic acid (Benckiser; Lot 7121) met the requirements of the European Pharmacopoeia and were used without further purification. Symmetrical and asymmetrical dimethyl citrates were synthesized from citric acid according to a literature procedure,[5] and had melting points of 119–124 °C [lit.[5] mp 122–124 °C] and 79–81 °C [lit.[5] mp 80–81 °C], respectively. Diazomethane was prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide (Aldrich-Chemie, Steinheim, FRG) in the usual manner. All other chemicals used were of reagent grade.

High-Performance Liquid Chromatography—The chromatographic system consisted of a pump (Constametric III; Laboratory Data Control, Riviera Beach, FL), an autosampler (WISP 710 B; Waters Associates, Milford, MA), a variable wavelength UV detector (Spectra Monitor III; Laboratory Data Control, FL), and an integrator (SP 4270; Spectra-Physics, San Jose, CA). A fraction collector (model 201; Gilson, Villiers Le Bel, France) was used for the isolation of the citrate esters of codeine. The analysis of the tablet extracts for low levels of esters 1–3, as well as for the tartrate 4, was performed with a reversed-phase column (μBondapak C_{18}, 10-μm particles, 150 × 3.9 mm; Waters Associates) using methanol:phosphate buffer (20:80), pH 4.5, containing 0.011 M sodium pentanesulfonate (HPLC grade; Fisons Scientific Apparatus, England) as the eluant. However, this system did not separate the esters 1–3. To achieve the desired resolution a Nova-Pak C_{18} column (5-μm particles, 150 × 3.9 mm; Waters Associates) was used. The eluant consisted of methanol:phosphate buffer (20:80), pH 3, ionic strength 0.1, containing 0.005 M sodium pentanesulfonate. With both systems, the flow rate was 1.0 mL/min, and the detection wavelength was 238 nm. A sample of tablet powder corresponding to 10 mg of codeine phosphate was dissolved in 50 mL of the eluant. The injection volume was 20 μL.

Spectrometry—Positive FAB/EI mass spectra were obtained with a double-focusing high-performance mass spectrometer (model ZAB-HF; VG Masslab, England) using glycerol as the matrix. Positive CI mass spectra of the methyl esters of the citrate esters of codeine were recorded with a low resolution instrument (model 2091; LKB, Sweden) using NH_3 as the reagent gas. Samples were introduced via a gas chromatograph (model HRGC 5300; Carlo Erba Strumentazione, Italy) using a homemade 8-m SE-54 fused silica capillary column. Injector and column were maintained at 290 °C, and the ion source was held at 270 °C. The tartrate ester of codeine was introduced via the heated direct inlet probe. The ^1H and ^{13}C NMR spectra were obtained at ambient temperature (unless otherwise stated) with a high-field NMR spectrometer (model FX 200; Jeol Ltd., Japan) operating in the pulsed Fourier transform mode at 199.5 and 50.1 MHz, respectively. The numbering of the acids is based on substitution in positions 1′ and 1″, respectively.

Solid-State Synthesis of Citrate Esters of Codeine (1–3)—Citric acid (0.8 g) and codeine phosphate hemihydrate (6.8 g; molar ratio 1:4) were thoroughly mixed in a mortar and then transferred to a weighing glass with a lid and kept at 90 °C for 4 d in the dark. The yield of esters, calculated from a liquid chromatography chromatogram, was ~40%. After dissolution in water (50 mL), the pH was adjusted to 8.7 with 5 M NaOH and unreacted codeine was extracted with 2 × 150 mL of methylene chloride. The water phase was filtered through a glass fiber filter and the pH was adjusted to 4.5. The solution was then evaporated under reduced pressure to ~5 mL and placed in a refrigerator overnight. The precipitate formed was removed by filtration, washed with water, and air dried.

The resulting material was separated on a 250 × 10 mm column packed with Nucleosil C_{18} (7-μm particles; Macherey–Nagel; Dueren, FRG), with acetonitrile:phosphate buffer (10:90), pH 6.5, ionic strength 0.05, as the eluant. In this system, two fractions of **1** and **3** were obtained. From the midfraction (mixture of **2** and **3**) **2** was isolated using an eluant of methanol:tetrahydrofuran:phosphate buffer (13:1:86), pH 3.4, ionic strength 0.05. The volumes of the collected fractions were reduced under reduced pressure to initiate precipitation. When the second eluant was used, the pH was adjusted to 5.5 before evaporation. The substances were recrystallized from methanol:water; mp of **1**: 158–160 °C (dec.); mp of **2**: 177–179 °C (dec.); and mp of **3**: 174–176 °C (dec.).

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Spectral Data—Positive FAB-MS of 1, 2, and 3: *m/z* 474 $(M + H)^+$; ^{13}C NMR: (D_2O, DCl) of 1: δ = 120.9 (C-1), 115.3 (C-2), 142.5 (C-3), 146.3 (C-4), 87.4 (C-5), 69.1 (C-6), 129.3 (C-7), 127.0 (C-8), 61.0 (C-9), 21.8 (C-10), 124.3 (C-11), 129.1 (C-12), 41.3 (C-13), 38.5 (C-14), 32.6 (C-15), 47.8 (C-16), 38.5 (CH_3-N), 56.8 (CH_3-O), 173.3 (C-1′ or C-5′), 43.4 (C-2′ or C-4′), 73.6 (C-3′), 43.2 (C-4′ or C-2′), 173.4 (C-5′ or C-1′), and 173.8 ppm (C-6′). (Contributions from the axial inverto isomer appeared at δ = 27.3, 31.1, 38.5, 41.9, 44.6, 90.0, and 133.0 ppm.)

Solid-State Synthesis of the Tartrate Ester of Codeine (4)—This substance was prepared in an analogous manner to the esters 1–3 using (2*R*, 3*R*)-2,3-dihydroxysuccinic acid and codeine phosphate in a 1:2 molar ratio. The product (10%) was recrystallized from water; mp 181–183 °C (dec.).

Spectral Data—Positive FAB-MS: *m/z* 432.176 $(M + H)^+$; calc. for $C_{22}H_{26}NO_8$ *m/z* 432.166; DI/+CI(NH_3)-MS: *m/z* (%) 387 (2), 370 (5), 342 (2), 315 (4), 301 (25), 300 (100), 298 (17), 285 (17), 284 (93), 283 (8), 282 (21), 60 (7), 58 (11); ^1H NMR (D_2O): δ ~2.15 (dm, H-15e), 2.39 (tm, H-15a), ~3.0 (m, H-10a), 3.07 (bs, NCH_3), 3.17 (m, H-16a), 3.29 (m, H-16e), 3.36 (m, H-14), 3.84 (d, J = 19 Hz, H-10e), 3.90 (s, OCH_3), 4.29 (m, H-9), 4.38 (d, J = 1.7 Hz, H-2″), 4.79 (d, J = 1.9 Hz, H-1″), 5.37 (d, J = 6.8 Hz, H-5), 5.43 (m, H-6), 5.65 (dm, J = 10 Hz, H-8), 5.83 (dm, J = 10 Hz, H-7), 6.86 (d, J = 8 Hz, H-2), 6.98 (d, J = 8 Hz, H-1), shift values are relative to methanol, δ_{CH_3} = 3.40 ppm; ^{13}C NMR (D_2O, 50 °C): δ = 121.6 (C-1), 116.2 (C-2), 143.2 (C-3), 146.7 (C-4), 87.7 (C-5), 69.2 (C-6), 130.0 (C-7), 127.3 (C-8), 61.7 (C-9), 21.7 (C-10), 124.7 (C-11), 129.3 (C-12), 41.7 (C-13), 38.8 (C-14), 32.9 (C-15), 48.3 (C-16), 41.7 (CH_3N), 57.7 (CH_3O), 173.6 (C-1″), 74.2 (C-2″ or C-3″), 73.3 (C-3″ or C-2″), 177.0 ppm (C-4″).

Preparation of the Methyl Esters 5, 6, and 7—Approximately 10 mg of each of the purified isomers of 1, 2, and 3 was dissolved in 5 mL of 0.1 M HCl. The solvent was evaporated and the residual material was dissolved in methanol:ether (1:3) and treated with an excess of diazomethane in ether. Unreacted diazomethane was removed by a stream of nitrogen. The dimethyl esters 5, 6, and 7 (from 1, 2, and 3, respectively) were purified by preparative thin-layer chromatography (Silica gel 60, precoated plates, 20 × 20 cm, 0.25 mm; Merck, Darmstadt, FRG) using 1,2-dichloroethane:methanol:formic acid (10:3:0.5) as the eluant. The dimethyl esters appeared at R_f = 0.34–0.44 and codeine appeared at R_f = 0.25. The bands corresponding to the dimethyl esters were scraped off and eluted with methanol. The solvent was removed by evaporation and then 5–7, in the form of formate salts, were converted to the bases by alkaline extraction (pH 8.5) with methylene chloride. After removing the solvent, oily products were obtained.

Spectral Data—+CI(NH_3)/GC-MS for dimethyl ester 5: *m/z* (%) 503 (0.72), 502 (0.88), 501 (1.49), 500 (0.62), 317 (5), 301 (21), 300 (100), 299 (10), 298 (12), 285 (9), 284 (43), 282 (17); for dimethyl ester 6: *m/z* (%) 503 (0.15), 502 (0.16), 501 (0.24), 500 (0.15), 343 (23), 342 (100), 341 (11), 340 (6), 301 (17), 300 (86), 299 (10), 298 (15), 285 (18), 178 (20); for dimethyl ester 7: *m/z* (%) 502 (0.2), 501 (0.34), 500 (0.1), 343 (21), 342 (82), 341 (11), 340 (9), 301 (22), 300 (100), 299 (12), 298 (16), 284 (24), 281 (25), 178 (16).

Partial Hydrolysis of the Methyl Esters 5, 6, and 7—Approximately 1 mg of each of the methyl esters 5, 6, and 7 was dissolved in 25 μL of tetrahydrofuran. Then, 50 μL of 0.1 M NaOH was added and the solutions were kept at room temperature for 30 min. Then, 0.5 mL of water and 0.5 mL of chloroform were added. After extraction, the chloroform layer was discarded and the pH of the aqueous phase was adjusted to 3. This phase was then extracted with 2 × 0.5 mL of ethyl acetate. The organic phase was evaporated to dryness and examined by thin-layer cochromatography, using the above-mentioned system. The symmetrical and asymmetrical dimethyl citrates appeared at R_f = 0.47 and 0.52, respectively.

Results and Discussion

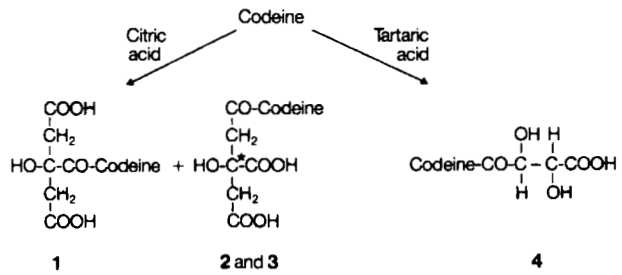
Liquid Chromatography—The presence of unknown degradation products in the analgesic combination containing citric acid was already noted after 3 months of storage. One additional peak of ~5% (calculated from codeine) was noted by HPLC (pH 4.5 phosphate buffer eluant) for samples stored at 25 °C. For samples stored at 37 °C, this increased to ~15%. We also observed that degradation was initially fast and leveled off on prolonged storage.

Preliminary investigations, including a compatibility study and hydrolysis of the HPLC fractions, indicated the possibility of ester formation from codeine and citric acid. Since three such esters can be formed (see Scheme I), it became necessary to modify the original liquid chromatographic system. Three well-separated peaks were observed using a pH 3 phosphate buffer (see Fig. 1).

Solid-State Synthesis—To prepare the monoesters by conventional methods would require several steps for unambiguous synthesis. Instead, a nonsolvolytic reaction was tested since this would support the degradation process taking place in the formulation. Careful mixing of the reactants and closing of the reaction vessel with a lid was found to have a positive effect on the reaction yield. The mixtures remained solid during the reaction, but some settling was observed, particularly during the formation of 4. At temperatures >90 °C, codeine was found to decompose extensively. Of the three citrate esters formed, 1 was always formed in the highest yield, although there was a variation in the relative amounts.

Since the (2*R*, 3*R*) form of tartaric acid was used, only one ester was formed. The yield of 4 was comparatively low (10%), but the synthetic procedure is simple and gives essentially homogeneous (by LC) material. The retention times of the synthetic substances were identical with those of the impurities in the formulations.

Spectroscopic Analysis—*Mass Spectrometry*—Direct molecular ion information for 1–4 was obtained from the FAB mass spectra only. However, DI/CI (NH_3) of 4 gave ions at *m/z* 387 (2%, M − CO_2) and 370 (5%, M − CO_2 − OH) which



Scheme I

Scheme I—Structures of esters formed from codeine and citric and tartaric acids, respectively.

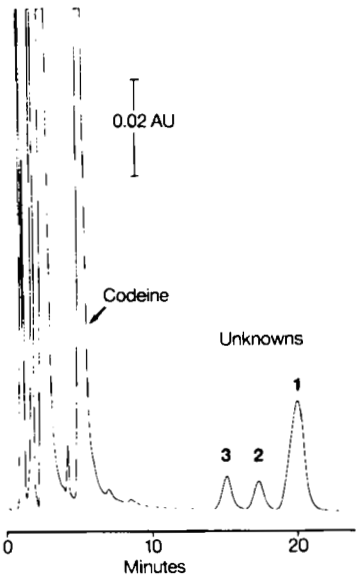


Figure 1—*Liquid chromatogram of an effervescent tablet after 3 months storage at 37 °C.*

is in accordance with the proposed structure. Structural information for 1–3 was obtained from the corresponding dimethyl esters 5–7, which could be injected via GC. Using CI (NH_3), clusters of ions were observed at *m/z* 502, with *m/z* 501 (quasimolecular ion − H) being most abundant. Of particular interest is the occurrence of an ion at *m/z* 342 (base-peak of 6, 82% of 7) which was absent in the spectrum of 5. A reasonable explanation is the formation of the fragment.

$$[CH_2{=}\underset{\displaystyle OH}{\underset{|}{C}} - \text{codeine}]^+$$

via a McLafferty rearrangement. This fragment could result from an unsymmetrically substituted citrate of codeine and supports the proposed structures of 2 and 3 (see Scheme I). Formation of such a fragment from 5 is less probable.

NMR Spectroscopy—The ^{13}C NMR spectra of esters 1 and 4 were assigned by comparison with shift data from codeine phosphate, literature values of citric and tartaric acids,[6] and from fully coupled spectra. Agreement with published spectra of codeine (in $CDCl_3$)[7] and morphine (in D_2O)[8] is excellent. The signals from the axial inverto isomer[8] were not assigned.

It is evident from the spectra that substitution has taken place at the C-6 position of codeine since the signal for carbon-6 is shifted downfield (~3 ppm) relative to that of codeine, whereas signals for carbon atoms C-5 and C-7 have moved upfield (3–4 ppm). Of particular interest in the spectra of 1 is the occurrence of dual signals for carbon atoms C-1′, C-5′, and C-2′, C-4′, respectively, despite symmetrical substitution. Steric effects due to hindered rotation may account for this observation. Thus, ^{13}C NMR alone is not very useful for unambiguous differentiation of the structures.

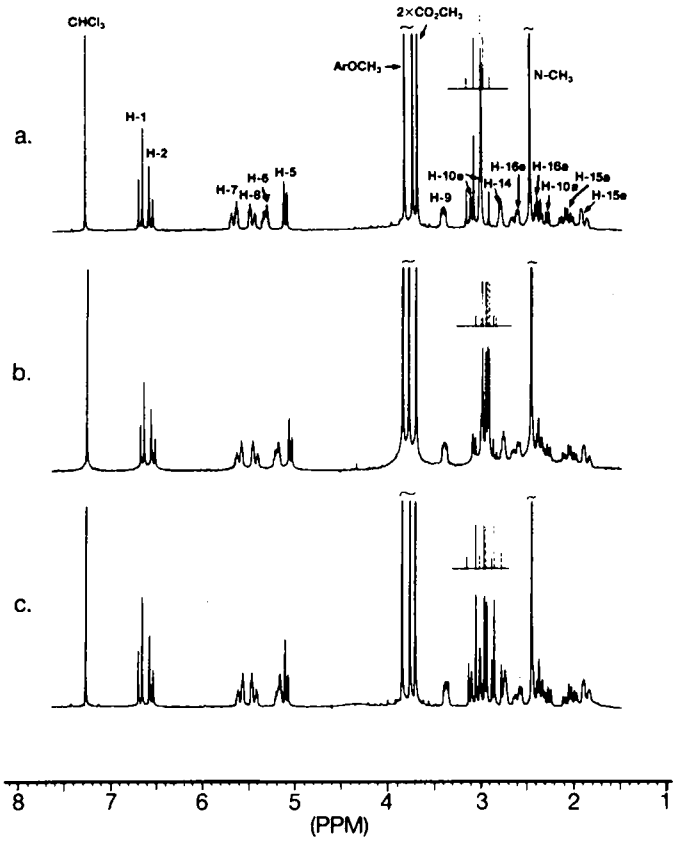
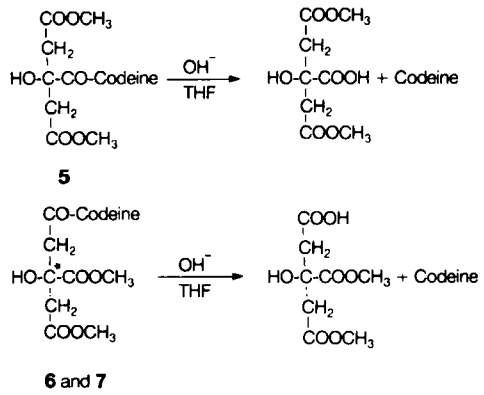


Figure 2—*The ^1H NMR spectra in $CDCl_3$ of methyl esters 5–7 (A–C). Shifts from Me_4Si using δ_{CHCl_3} = 7.26.*



Scheme II

Scheme II—Selective alkaline hydrolysis of dimethyl codeine citrates.

The proton NMR spectra of the methyl esters 5, 6, and 7 are presented in Fig. 2. The assignments shown were based on literature data,[9,10] decoupling experiments, and homoscalar correlated 2D-NMR spectra (COSY). Spin–spin coupling constants for selected protons of morphine have recently been presented in a 600 MHz study.[11] The proton in position 6 of the methyl ester 5 is shifted from δ 4.16 to 5.30 ppm (relative to codeine), whereas the corresponding protons in the esters 6 and 7 are less shielded (δ 5.18 and 5.14 ppm, respectively). Differences are also observed for the 2′- and 4′-methylene protons, which appear in the region δ 2.8–3.1 ppm as AB quartets (J_{AB} = 15.4–16.1 Hz) in all cases except for one of the methylene groups of ester 5 where J = 0 (see Fig. 2 and inserted coupling patterns).

Final structural proof—All esters (1–4) yield codeine and the respective acids after mild alkaline hydrolysis. Rigorous proof of the structures of the citrates was obtained by selective hydrolysis of the dimethyl esters 5–7. As the rate of cleavage of the codeine ester bond in position 6 is faster than that of the methyl ester bonds, it was possible to differentiate between the symmetrical (1) and asymmetrical (2, 3) codeine citrates (see Scheme II).

The products, codeine and symmetrical and asymmetrical dimethyl citrates, were subsequently identified by TLC. The experiment shows that 1 (Fig. 1) most likely is the symmetrical citrate ester of codeine, and that 2 and 3 are the diastereomeric esters. The absolute configurations of 2 and 3 have not been determined.

During the solid-state reaction of codeine with the two different dimethyl citrates, the corresponding esters 5, 6, and 7 were also formed. However, the yields were low (≤1%), and some isomerization took place when the symmetrical dimethyl citrate was used as starting material.

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