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Synthesis and structure elucidation of a new isoquinolinium inner salt

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ABSTRACT

Papaverine is a drug that can be easily oxidized to papaverinol, papaveraldine and to recently discovered 2,3,9,10-tetramethoxy-12-oxo-12*H*-indolo[2,1-*a*]isoquinolinium chloride. In a strong alkaline medium the spectroscopic properties of this latter compound are modified indicating formation of a new compound. The isolation and structure elucidation of this compound as 2-(2-carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt are reported.

Papaverine **1** is an isoquinoline alkaloid that is found in opium.[1] In medical therapy its hydrochloride and sulfate salts are used and it is unstable if exposed to oxygen and UV light. Oxidation of **1** leads to products such as papaverinol, papaveraldine and the recently discovered 2,3,9,10-tetramethoxy-12-oxo-12*H*-indolo[2,1-*a*]isoquinolinium chloride **2**.[2,3] Compound **2** inhibits telomerase and polymerase Taq activity[4] and its cytotoxic behaviour has been investigated against breast cancer, malignant melanoma, lung adrenocarcinoma, laryngeal cancer and gastric cancer cell lines.[5] In contrast to **1**, compound **2** is tetracyclic. The characteristic features of the structure of **2** are the presence of a carbonyl group and positively charged nitrogen bonded to a substituted phenyl ring (Fig. 1). The above-mentioned oxidation products of **1** are found on storage of its injection solutions, which become first yellowish, then brownish in colour.[6]

A brown methanol solution of **2** is discoloured upon addition of aqueous NaOH solution which also results in UV spectral changes. The absorption maxima of **2** in methanol solution are hypsochromically shifted from $\lambda_{max} = 310$ nm ($\lg \varepsilon = 4.74$) and $\lambda_{max} = 398$ nm ($\lg \varepsilon = 4.10$) to $\lambda_{max} = 256$ nm ($\lg \varepsilon = 4.77$) and $\lambda_{max} = 322$ nm ($\lg \varepsilon = 4.17$) when NaOH is added as a result of formation of ring-opened compound **3** (Fig. 2).

The structure of compound **3** was deduced from mass spectrometric experiments. The electron impact mass spectrum (EI-MS) of **3** gave a molecular ion at *m*/*z* 369. The electrospray ionization mass spectrum (ESI-MS) was characterized by a pseudomolecular ion $[M+H^+]$ at *m*/*z* 370 and the molecular formula is based on HREI-MS of the $[M^+]$ ion peak at *m*/*z* 369.12205, calculated for $C_{20}H_{19}NO_6$; 369.12124 (Δ −2.2 ppm). The new product was identified as 2-(2-carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt **3**; molecular formula: $C_{20}H_{19}NO_6$.[7] On addition of hydroxide, ring opening occurs at C-12 of compound **2** via the mechanism proposed in Scheme 1.

The structure of compound **3** was confirmed by NMR experiments. Examination of the ^{1}H NMR spectrum of **3** obtained in methanol-d_4 (TMS as internal standard) revealed clearly the presence of seven aromatic protons and twelve protons due to the methoxy groups (Table 1). The ^{1}H NMR spectrum was also recorded in DMSO-d_6 and 19 protons were again observed. The aromatic protons were assigned to the isoquinoline ring and a phenyl substituent. Four three-proton singlets were assigned to the four methoxy groups: two isoquinoline (δ 4.13, C-6; δ 3.98, C-7) and two phenyl (δ 3.96, C-4′; δ 3.90, C-5′). The ^{1}H, ^{13}C HSQC spectrum confirmed the presence of seven aromatic protons.

The negatively charged carbon of the carboxyl group is deshielded (δ 170.29) in the ^{13}C NMR spectrum. According to the literature data the $-COO^-$ carbon appears at δ 169.8 in reticulatate and at δ 164.1 in 14-bromoreticulatate in methanol-d_4.[8]

The ^{1}H, ^{13}C HMBC spectrum showed no correlation between H-3 and the $-COO^-$ carbon. However, protons H-3′ and H-6′ did correlate with the carboxylate carbon at δ 170.29. Additional correlations are shown in Table 1.

The NOESY coupling between H-1 and H-8 confirms the presence of a hydrogen at δ 9.47 on the isoquinoline ring and this was also proved by the COSY LR (LR—long range) correlation of H-1 with H-3, H-4 and H-5. NOESY and COSY LR correlations are also presented in Table 1.

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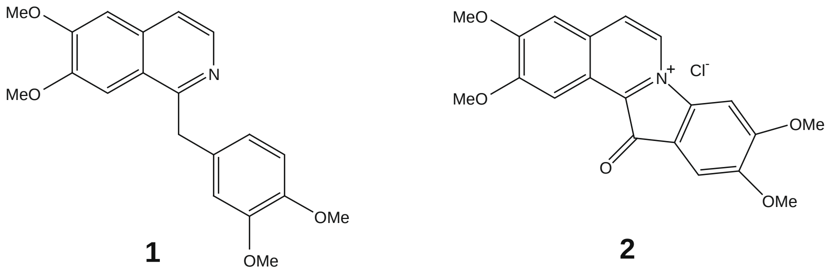


Figure 1. The structure of papaverine **1** and 2,3,9,10-tetramethoxy-12-oxo-12*H*-indolo[2,1-*a*]isoquinolinium chloride **2**.

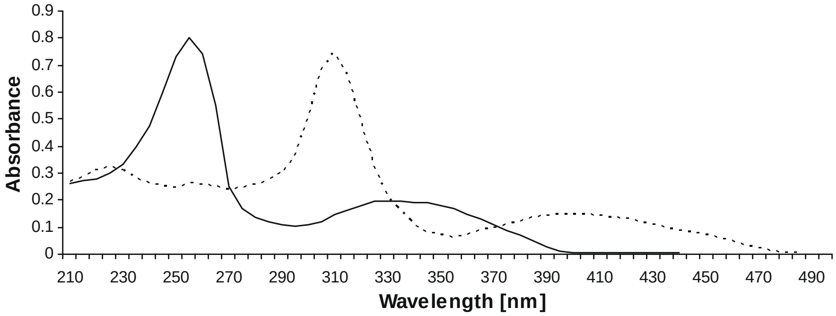
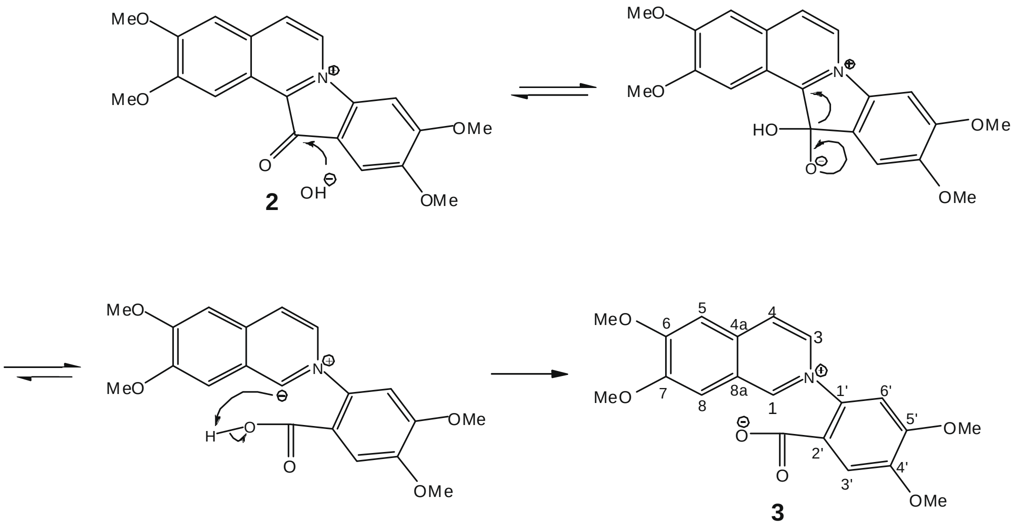


Figure 2. The UV–vis spectra of compounds **2** (dashed line) and **3** (solid line) in methanol.



Scheme 1. Mechanism of the formation of 2-(2-carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt **3**.

The chemical shift of the nitrogen in the ^{15}N NMR spectrum (−178.3; nitromethane scale) clearly shows that it is positively charged.[9] The HMBC ^{1}H–^{15}N NMR spectrum recorded in DMSO-d_6 showed couplings to the signals at δ 8.34 and δ 8.16 (H-3 and H-4), to the two singlets at δ 7.15 and δ 7.58 (H-6′ and H-3′) and to the doublet at δ 9.47 (H-1). This confirms that the isoquinoline nitrogen is linked to the phenyl ring carbon.

The proton at δ 9.47 is shifted downfield as a result of the positively charged nitrogen and negatively charged carboxyl group. Similar values can be found in reticulatate (δ 9.21) and 14-bromoreticulatate (δ 9.41).[8] These protons are *ortho* with respect to the positively charged nitrogen.

The NMR data also confirm the presence of isoquinoline and phenyl rings and are similar with the NMR data for compound **2**.[3]

Peaks in the IR spectrum due to antisymmetrical and symmetrical stretching at 1599 cm^{-1} and 1398 cm^{-1}, respectively, are characteristic of the –COO^{-} group.[10]

Stable zwitterions can also be found among 2,4,6-triphenylpyridinum derivatives.[11]

Furosemide is a drug that can also be oxidized to zwitterions. Its chemical oxidation leads to 4-chloro-2-(3-hydroxypyridinium-1-yl)-5-sulfamoylbenzoate which has an inner salt formula.[12] These compounds possess a positively charged nitrogen and the –COO^{-} group *ortho*[5] to the carbon bonded to nitrogen in the isoquinoline ring.

Walterová et al.[13] reported that papaverine derivatives exist as pseudobases in alkaline solutions. The hydroxy groups bond to the isoquinoline ring at position 1 as was confirmed by ^{1}H NMR analysis. Our study confirms the observations of Fretz et al.[14] who ob-

Table 1

^{15}N (30 MHz), ^{13}C (75 MHz), ^{1}H (300 MHz), COSY LR, HMBC and NOESY data for 2-(2-carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt **3** in methanol-d_4

No.	^{15}N (δ)[a]	^{13}C (δ)	^{1}H (δ, Hz)	COSY LR[c]	HMBC (^{1}H–^{13}C)	HMBC (^{1}H–^{15}N)[d]	NOESY
1		147.5	9.47 d (1.5)	3, (4), 5	1′, (3), 4a, 8, 8a	(2)	6′, 8
2	−178.3 (−181.9)[b]					(1), 3, (3′), 4, 6′	
3		136.27	8.34 dd (6.8; 1.5)	4	1,1′, 4, 4a, (6), (8a)	2	6′
4		123.13	8.16 d (6.8)	3, 8	(1), 3, 5, (8), 8a	2	5
4a		137.33					
5		106.38	7.64 s	3,6-OMe	(1), 4, 6, 7, 8a		4, 6-OMe
6		159.97					
7		154.35					1
8		108.29	7.57 s	4, 7-OMe	1,(4), 4a, (5), 6, 7		7-OMe
8a		125.09					
1′		136.15					
2′		128.24					
3′		114.35	7.58 s	4′-OMe, 6′	1′, 2′, 2′-COO$^-$, 4′, 5′, 6′	(2)	4′-OMe
4′		151.32					
5′		151.49					
6′		110.74	7.15 s	3′,5′-OMe	1′, 2′, 2-COO$^-$, (3′), 4′, 5′	2	5′-OMe
6-OMe		57.52	4.13 s	5	5, 6		5
7-OMe		57.00	3.98 s	8	7, 8		8
2′-COO$^-$		170.29					
4′-OMe		56.54	3.96 s	3′	3′, 4′		3′
5′-OMe		56.95	3.90 s	6′	5′, 6′		6′

[a] Chemical shift obtained from the ^{1}H–^{15}N HMBC NMR and referenced to nitromethane.

[b] Chemical shift recorded in DMSO-d_6.

[c] Weak signals in parentheses.

[d] Spectrum recorded in DMSO-d_6.

served that fascaplysin, on treatment with a solution containing OH$^-$ ions, underwent a change in its colour indicative of its conversion to reticulatate.[7,14] In the case of **2** the tetracyclic structure is converted into zwitterionic product **3** and the UV spectrum is shifted to a shorter wavelength.

Compound **2** was synthesized by irradiating a 0.3% (w/v) chloroform solution of papaverinol with a low-pressure mercury lamp at 254 nm for 4.5 h.[3] The crude material dissolved on boiling in methanol and crystallized as a black powder; yield up to 40%.

Compound **3** was obtained by dissolving **2** in a 0.4% aqueous NaOH solution with heating for 2 h at 60 °C. The solvent was evaporated and the residue was dissolved in $CHCl_3$–CH_3OH (1:1) mixture (yield of crude product was 15%). The product was isolated by column chromatography on aluminium oxide (ECO-CHROM, Germany), mobile phase: reagent grade $CHCl_3$, $CHCl_3$–CH_3OH (20:1, 10:1, 5:1, 1:1 v/v) and finally reagent grade CH_3OH. The product was observed on the column as a white fluorescent band using a UV$_{365}$ lamp and was separated, washed with water and chloroform and dried (yield of pure **3** = 10%). The purity was confirmed by TLC on aluminium oxide (POLYGRAM, MACHEREY-NAGEL, Germany) using chloroform–methanol (1:1; v/v) as the mobile phase; R_f = 0.88.

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