A novel route to new carbonyl derivatives of cinchonine and cinchonidine †

Guy Dodin,*[a] Christine Cordier,[a] Laurence Menager,[a] Aicha Bourzegue[a] and Jean-Claude Blais[b]

[a] *ITODYS, Université Denis Diderot, CNRS URA 34, 1 rue Guy de la Brosse, 75005 Paris, France*
[b] *Laboratoire de Chimie Structurale et Biologique, Université P. et M. Curie, 4 place Jussieu, 75005 Paris, France*

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Cinchonine and cinchonidine are readily transformed to their corresponding *N*-oxides by the action of MMPP in acetonitrile–bicarbonate buffer. Methylation of the *N*-oxides by iodomethane in dichloromethane leads, under mild conditions, to the formation of a unique enol stabilized by intramolecular hydrogen bonding. In water the enol form tautomerizes to its parent keto derivatives. The reaction has been extended to quinine and quinidine.

The *Cinchona* alkaloids have long been known for their broad spectrum of biological and pharmaceutical properties (as antimalarials, antiarrythmics, Na^+-channel blockers, *etc.*). More recently, members of this family (cinchonine and cinchonidine) have shown their efficacy in restoring sensitivity towards antitumor drugs in cells expressing the multidrug resistance (MDR) phenotype.[1] In the field of chemistry, *Cinchona* derivatives have found a large domain of application as chiral catalysts in asymmetric synthesis.[2] On the other hand, *N*-oxides, arising from oxidation of both tertiary aliphatic amines and heteroaromatic bases constitute a broad family of useful chemical precursors in organic synthesis as well as biologically active compounds. In this context, we have become involved in the preparation of *N*-oxides of *Cinchona* derivatives with particular emphasis on $N_{\text{quinuclidine}}$-oxides where the aromatic nitrogen is left available for quaternarization (meant to increase water solubility and electrostatic interaction with oligonucleotides presently under study in our laboratory). Hydrogen peroxide readily oxidizes saturated tertiary nitrogens but, in general, fails to give aromatic *N*-oxides. On the other hand, use of organic peracids (peracetic acid, 3-chloroperbenzoic acid) leads to rapid formation of both types of *N*-oxides. Here we show that magnesium monoperoxyphthalate (MMPP) offers a convenient route to *N*-oxides involving the quinuclidine moiety in the *Cinchona* derivatives.

Treatment of cinchonidine, **1**, and cinchonine, **2**, in buffered water–acetonitrile with one equivalent of magnesium monoperoxyphthalate (MMPP) at room temperature leads to the rapid formation of $N_{\text{quinuclidine}}$-oxides (compounds **3** and **4** respectively, see Experimental section). No epoxidation of the ethylenic bond occurs, as seen from the typical resonance lines in the 5–5.8 ppm region of the proton NMR spectrum, also present in the starting molecules (Table in Supplementary Material).[3] Addition at the quinuclidine nitrogen atom is consistent with an expected faster reaction rate at this site, as compared to addition at the aromatic nitrogen atom, and with the downfield chemical shifts experienced by C(2) and C(6) [whereas the C(2′) and C(9′) resonances are only slightly affected]. ^{14}N and ^{15}N NMR also show oxygen addition at the quinuclidine moiety. It is worth noting that the H(9) proton is strongly deshielded in the *N*-oxides with respect to the starting alkaloids (Tables in Supplementary Material).

Increasing MMPP concentration and reaction time leads to addition of a second oxygen atom at the aromatic ring nitrogen. Studies on the dioxygenated species are not reported here.

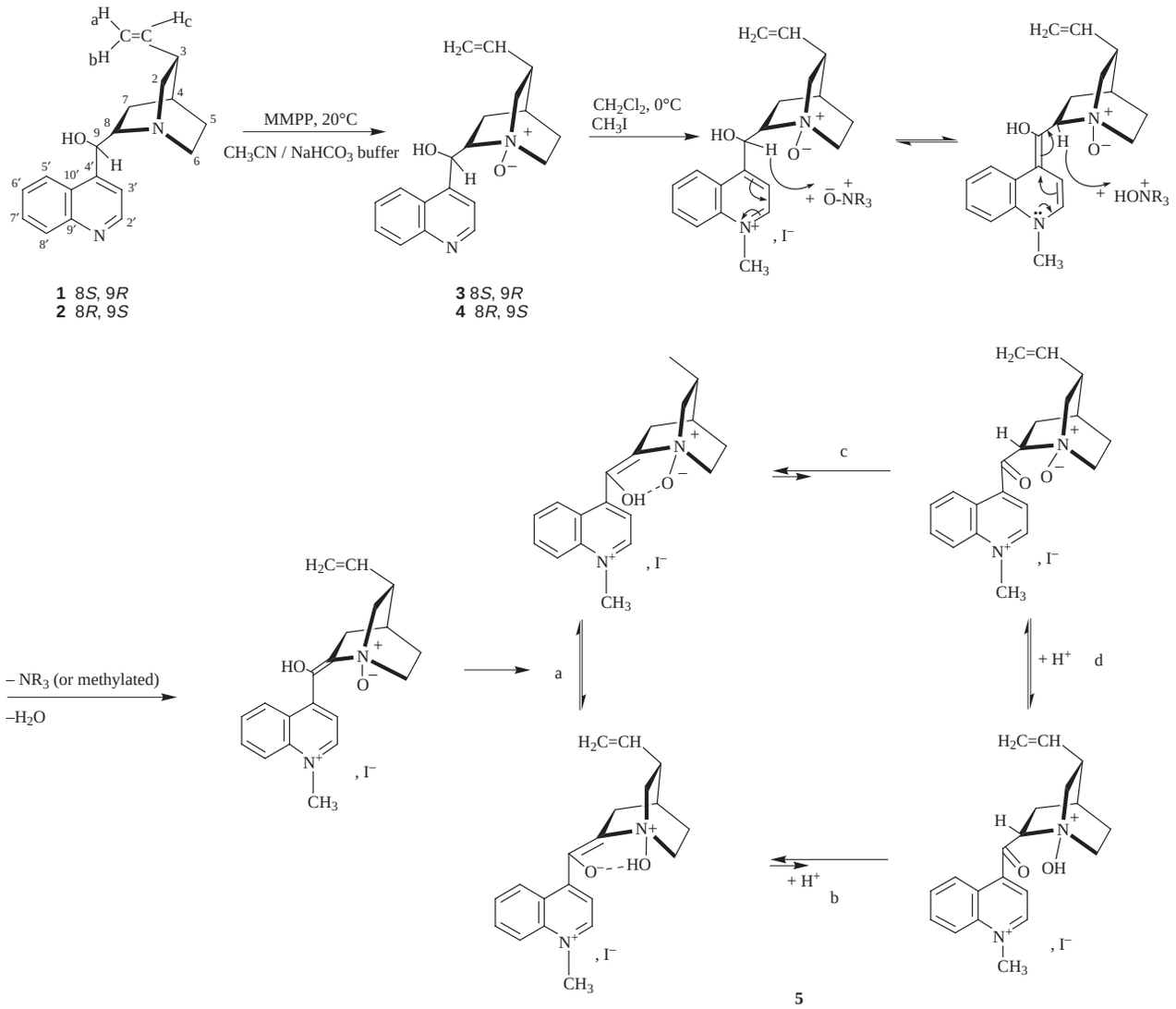
Treatment of either **3** or **4** by iodomethane at 0 °C leads to the *same water-soluble derivative* (**5**) as shown by the ^1H and ^{13}C NMR spectra in DMSO-d_6 (see Experimental section; Tables in Supplementary Material and Fig. 1). Remarkably, the resonances of carbons C(9) and C(8) are shifted strongly downfield and their state of substitution is changed, going from tertiary to quaternary (see Fig. 1c, the SEFT spectrum of **5**). This is good evidence for formation of a double bond between C(8) and C(9) conjugated with the aromatic ring. Moreover, reaction with CH_3I induces a downfield shift of the H(2′) proton,[4] similar to that observed in cinchonine and cinchonidine dimethiodides (Tables in Supplementary Material) and in quinoline methiodide, the aromatic moiety of the alkaloids (data not shown). ^{13}C NMR in DMSO-d_6 shows a 10 ppm upfield shift of the resonance of C(9′) and the resonance of the methyl carbon at 45 ppm (as in the dimethylcinchoni(di)nium and the quinolinium cations). This indicates that methylation of the *N*-oxides takes place at the aromatic nitrogen atom. Formation of the ammonium iodide (whereas C- or O-methylation would lead to a neutral species), is further confirmed by H_2O_2 oxidation of **5** followed by iodine titration with sodium thiosulfate.

Together with the water-soluble **5**, the reaction mixture contains a derivative sparingly soluble in water and highly soluble in dichloromethane identified from its ^1H, ^{13}C NMR and mass spectra as the starting *N*-oxides. We were not able to identify other species present in the reaction medium.

A blank run shows no reaction of the *N*-oxides under the experimental conditions in the absence of iodomethane.

Dramatic changes are observed when now the NMR spectra of **5** are run in buffered D_2O (pH 6.86) (Fig. 1d). The resonance of C(9) is strongly shifted from 149 ppm in DMSO to 192 ppm, a ^{13}C chemical shift characteristic of carbonyl derivatives. Carbon C(8) becomes deshielded by 28 ppm and its state of substitution is altered, going from quaternary to tertiary (Fig. 1d). Most of the resonance lines are duplicated. Significantly, when the sample in D_2O is freeze-dried and the pellet subsequently dissolved in DMSO-d_6 the previous spectrum (Fig. 1c) is fully restored. The NMR run in D_2O at pH 9.18 shows slow progressive decomposition of **5** at this pH. How-

† Supplementary data are available for this paper (SUPPL. NO. 57441, pp. 6) from the British Library. For details of the Supplementary Publications Scheme, see 'Instructions for Authors', *J. Chem. Soc.*, *Perkin Trans. 1*, available *via* the RSC Web page (http://www.rsc.org/authors).



Scheme 1

ever, the carbonyl resonance at 190 ppm which is not observed at pH 9.18 is present again in the spectrum when the pH is readjusted to 7 (spectra not shown).

Formation of **5** results from an overall oxidation process. Since the reaction is carried out in oxygen-free solutions, we postulate that the oxidant must be the *N*-oxide itself acting both as proton and hydride acceptor as does *N*-methylmorpholine *N*-oxide in *N*-methylmorpholine *N*-oxide–osmium tetroxide-catalyzed hydroxylation of olefins.[5] However, despite our efforts, we were not able to detect a reduced form of the starting *N*-oxide, the *Cinchona* alkaloid itself (referred to as NR_3 in Scheme 1), which should be present in the reaction mixture at a proportion similar to that of **5**. Since the reduced species is not detected in the NMR spectra of the crude extracts, we conclude that it is subjected to extensive degradation, possibly along the Hofmann exhaustive methylation pathway. The formation of the same cationic moiety when the *N*-oxides are treated either with dimethyl sulfate or iodomethane rules out occurrence of an electron transfer process involving iodide or iodine.

The following reaction mechanism is advanced (see overall reaction pathway in Scheme 1). Methylation of the aromatic nitrogen atom produces an electron sink that increases the acidity of the C(9) hydrogen which is then abstracted by a second *N*-oxide moiety. The marked downfield shift shows that the H(9) proton was already labile in the *N*-oxides. The nitrogen atom in the protonated *N*-oxide may then accept a hydride from C(8) thus leading to loss of a water molecule and formation of the alkaloid NR_3 (see Scheme 1) which is subsequently degraded by extensive methylation in the presence of the *N*-oxide as oxidant. The bicyclic structure of the quinuclidine is conserved in **5** since its rupture (as in a Cope reaction[6]) would result in the observation of tertiary C(8) in the SEFT ^{13}C spectrum. Proton and hydride transfer leads to an enol (or enolate) stabilized by hydrogen bonding with N–O (unprotonated or protonated respectively) (step **a** in Scheme 1). The equilibria **b** and **c** in Scheme 1 are strongly shifted in favor of the ethylenic species in DMSO and they are likely to account for the single enol observed starting either from **3** or from **4**.

The change of the UV spectrum of **5** with pH in water is attributed to the protonation of the *N*-oxide (step **d** in Scheme). The unusually high pK_a (proton gained; 8.12 whereas p*K* values of *N*-oxides are commonly found around 5) reflects the strong proton acceptor power of N–O in the methiodide *N*-oxides.

^{13}C NMR in D_2O at neutral pH shows that the equilibria **b** and **c** are now favoring the keto isomers, as a consequence of rupture of the hydrogen bond [see frequency shifts and carbon substitution in the NMR spectra (Fig. 1d)]. The duplication of the resonance lines suggests similar populations of two isomers where carbon C(8) adopts the *R* or *S* configuration.

The ammonium moiety of **5** is likely to bind to the phosphate groups of DNA and, if it can become imbedded into the core of the duplex, it will be stabilized as the strongly nucleophilic enolate that may react with specific sites of DNA bases such as C(6) and C(4) of pyrimidines. A preliminary study has shown that compound **5** is responsible for strand scission in plasmid pBr322 DNA.

The procedure that has allowed synthesis of new carbonyl

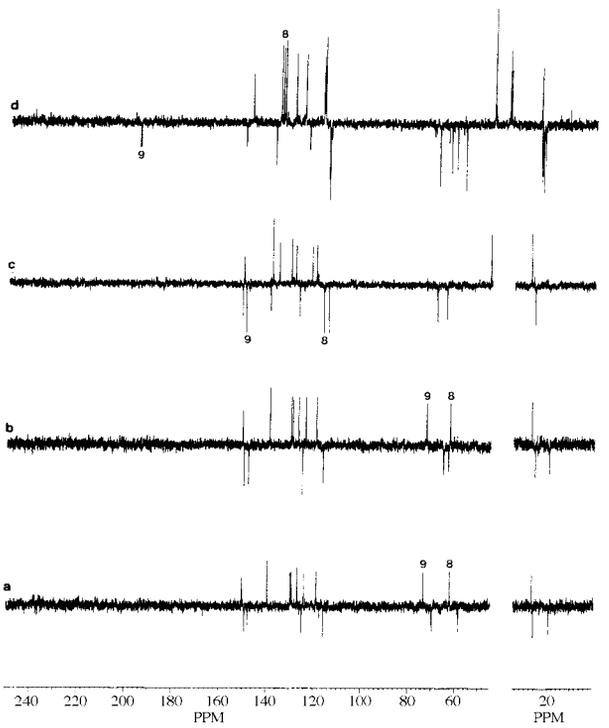


Fig. 1 Spin echo Fourier transform (SEFT) ^{13}C NMR spectra in DMSO-d_6 (a–c) and in D_2O, pH 6.86 (d). Above base line: resonance lines of primary and tertiary carbons; below base line: resonance lines of secondary and quaternary carbons. (a) Compound **3**; (b) compound **4**; (c) compound **5** (in DMSO-d_6); (d) compound **5** (in D_2O, NBS buffer, pH 6.86). Only the carbons bridging the quinuclidine and quinoline rings are marked [C(8) and C(9)]. For assignment of all resonances see Tables in Supplementary Material.

derivatives of cinchonine and cinchonidine has been successfully extended to quinine and quinidine (data not shown). It is likely to be of general applicability in the *Cinchona* alkaloid family.

Experimental

Synthesis of 3 and 4

590 mg (2×10^{-3} mole) of cinchonidine or cinchonine (Sigma) were suspended in 100 ml of an aqueous sodium bicarbonate 0.3 M–acetonitrile (70:30) mixture. 1 g of magnesium monoperoxyphthalate (MMPP) (2×10^{-3} mole) dissolved in 20 ml of the bicarbonate–acetonitrile buffer was added dropwise over 10 min at room temperature to the stirred suspension of the alkaloid. The progress of the reaction was followed by TLC [silica gel; eluent: aqueous 0.15 M ammonium acetate–acetonitrile (50:50)]. After 2 h the aqueous solution was washed with dichloromethane. Evaporation of the dried organic phase yielded 400 mg of **3** or **4** as a white powder homogeneous on TLC. Compound **3** or **4** was dissolved in acetonitrile and applied to a silica gel column and eluted with acetonitrile. Alternatively, crystalline samples were readily obtained from a dichloromethane solution of **3** or **4** allowed to stand in a closed vessel containing dimethyl ether. Mass spectroscopy (chemical ionization) *m/z* (M + 1) = 311 (calc. 311.39). Though **3** and **4** were introduced as pure compounds (as shown by TLC and NMR) significant departure of the oxygen atom occurs under the experimental conditions. Easy loss of O is observed in *N*-oxides[6] (Fig. S1 in Supplementary Material).

^1H NMR (aromatic and ethylenic) 200 MHz, DMSO-d_6; δ **3**: 8.9 (2′-H, d, 1H), 8.5 (5′-H, d, 1H), 8 (8′-H, d, 1H), 7.8 (7′-H, t, 1H), 7.7 (3′-H, d, 1H) 7.6 (6′-H, t, 1H), 6.7 (9-H, s broad, 1H), 5.8 (H_c, m, 1H), 5 (H_a,H_b, m, 2H). **4**: 8.9 (2′-H, d, 1H), 8.2 (5′-H, d, 1H), 8 (8′-H, d, 1H), 7.7 (7′-H, 3′-H, m, 2H), 7.3 (6′-H, t, 1H), 7 (9-H, s broad, 1H), 6.1 (H_c, m, 1H), 5.2 (H_a,H_b, m, 2H). ^{13}C SEFT NMR 200 MHz, DMSO-d_6 and D_2O: Fig. 1 and Table in Supplementary Material.

Synthesis of 5

In a typical experiment, a 20 ml dichloromethane solution of 310 mg of **3** or **4** (10^{-3} mole) and 0.3 ml of iodomethane (5.10^{-3} mole) at 0 °C was thoroughly deoxygenated by extensive argon bubbling. The progress of the reaction at 0 °C was monitored by TLC (silica gel; eluent: aqueous ammonium acetate 0.15 M–acetonitrile) which showed progressive accumulation of a polar species and corresponding decrease of the starting derivative. After 5 days the solvent and excess iodomethane were evaporated. The 390 mg crude pellet was redissolved in dichloromethane and the solution washed with water. The organic phase contained 210 mg of a crude material from which 100 mg of a white solid, identified as the starting *N*-oxide from mass spectrometry and NMR, were recovered after the purification step (elution by buffered acetonitrile from a silica gel column). Colored species could not be eluted from the column.

Freeze-drying of the aqueous solution, dissolution of the pellet in dichloromethane and addition of dimethyl ether led to precipitation of 150 mg an orange solid giving 120 mg of pure **5** after chromatography (conditions as above). Comparison of the ^{13}C and proton NMR spectra of the water- and dichloromethane-soluble material run before and after the chromatography step showed that no other abundant species was present in the crude extracts. Dimethyl sulfate has also been used as methylating agent in dichloromethane at 0 °C. The ^{13}C NMR spectrum of the water-soluble fraction of the reaction products unambiguously showed formation of **5** present together with another derivative, in a roughly similar proportion, and whose separation was impaired by the similarity of their chromatographic features. Hence, iodomethane is preferred over dimethyl sulfate in the synthesis of **5**. The MALDI-TOF mass spectrum of the aqueous phase and of the organic phase compounds showed peaks respectively at *m/z* 323.37 (calc. 323.39) (the mass of the cationic moiety of **5**) and at *m/z* 311.31 (the mass +1 of the *N*-oxide). Though the derivatives are introduced as pure compounds, significant mass peaks resulting from loss of oxygen are observed at *m/z* 307.36 and 295.3 respectively (see the mass spectrum of **5**, Fig. S2 in Supplementary Material).

^1H NMR 200 MHz, DMSO-d_6, δ: 9.5 (2′-H, d, 1H), 8.6 (5′-H, d, 1H), 8.5 (8′-H, d, 1H), 8.3 (6′-H, t, 1H), 8.1 (3′-H, 7′-H, m, 2H), 5.8 (H_c, m, 1H), 5.2 (H_a,H_b, m, 2H). ^{13}C NMR, 200 MHz, DMSO-d_6 and D_2O: Fig. 1 and Tables in Supplementary Material. The pK_a (proton gained) of **5** in H_2O (8.12) was measured at 25 °C from the changes of the UV spectrum with pH.

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