Stereochemistry of Bisbenzylisoquinoline *N*-Oxides. Calafatine 2α-*N*-Oxide and Calafatine 2β-*N*-Oxide

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The diastereoisomeric alkaloids calafatine 2α-*N*-oxide (2) and calafatine 2β-*N*-oxide (3) have been obtained from *Berberis buxifolia* Lam. (Berberidaceae). The stereochemistry at the *N*-oxide centres was determined by n.m.r. nuclear Overhauser difference studies.

The bisbenzylisoquinolines represent one of the largest subgroups within the isoquinoline alkaloids.[1a,b] Among the nearly 250 bisbenzylisoquinolines known, six *N*-oxides have been recognized, namely isogilletine *N*-oxide,[1b] tiliacorinine 2′-*N*-oxide,[2] funiferine 2-*N*-oxide, [1a] *N*-oxy-2′-isotetrandrine,[1b] tetrandrine *N*-2′-oxide,[1a] and hernandezine *N*-oxide.[1b] In none of these instances, however, has the stereochemistry of the *N*-oxide function been determined.

Our attention was focussed on this problem when, in the course of an investigation of the alkaloids of *Berberis buxifolia* Lam. (Berberidaceae) collected near the town of Punta Arenas, in Chilean Patagonia, we isolated two new amorphous bisbenzylisoquinoline *N*-oxides with markedly different t.l.c. R_F values.

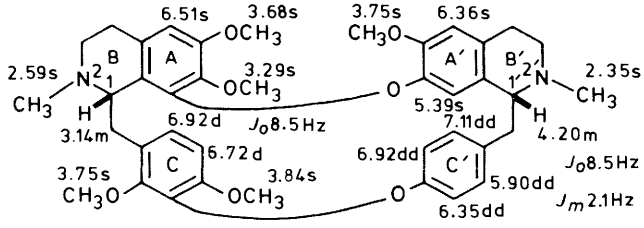
Previous studies of *B. buxifolia* had yielded the bisbenzylisoquinoline (−)-calafatine (1),[3,4] $C_{39}H_{44}N_2O_7$, whose stereochemistry at C-1 and C-1′ had been elucidated through sodium in liquid ammonia reduction to furnish two optically active monomeric benzylisoquinolines.[4]

Both of our new *N*-oxides analyzed for $C_{39}H_{44}N_2O_8$, giving weak molecular ions *m/z* 668, so that they incorporate one oxygen more than calafatine (1). In each case, the molecular weight was confirmed by chemical ionization mass spectroscopy. Zinc in hydrochloric acid reduction of either *N*-oxide afforded (−)-calafatine (1). The structural problem was thus to define to which nitrogen atom of (−)-calafatine the oxide oxygen was bonded in each instance, and then to determine the stereochemistry of the *N*-oxide function. We were particularly interested at this stage in setting up a standard approach by which the stereochemistry of the other known bisbenzylisoquinoline *N*-oxides referred to in the opening paragraph could also be elucidated.

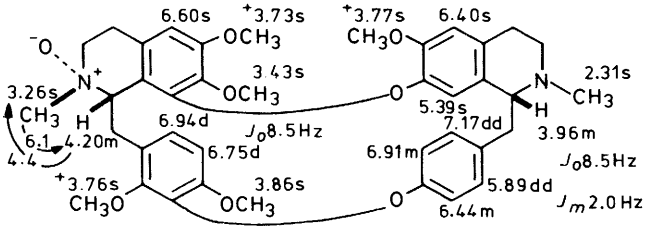
The n.m.r. spectrum of our first *N*-oxide dimer, calafatine 2α-*N*-oxide (2) has been summarized around expression (2). Comparison of the chemical shifts with those of calafatine (1) indicate that it is the protons associated with rings A and B of the *N*-oxide (2) that are shifted downfield, while the shifts for the protons of rings C, A′, B′, and C′ remain essentially unchanged. In particular, the protons of the oxygenated *N*-methyl group of (2) are found downfield at δ 3.26.

The mass spectrum of the *N*-oxide dimer (2) corroborated this assignment for the location of the *N*-oxide. The presence of ion *m/z* 222, $C_{12}H_{16}NO_3$, representing species (4), clearly indicates that it is the nitrogen atom of ring B that exists as an *N*-oxide.

Turning now to the second dimer, calafatine 2β-*N*-oxide (3), the n.m.r. spectrum of this species again indicates that it is the protons associated with rings A and B that have been shifted downfield by comparison with the mother dimer calafatine (1). The protons of the methyl group linked to the *N*-oxide are now even further downfield than was the case with *N*-oxide dimer (2), being located at δ 3.41.



(1)



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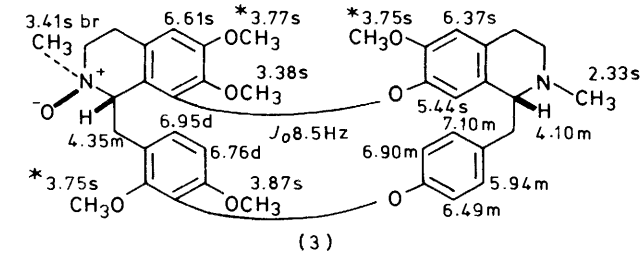
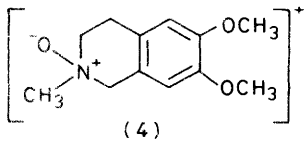


(3)

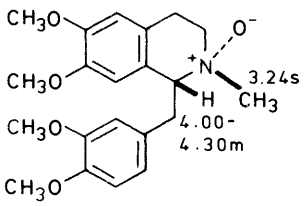
Furthermore, the mass spectrum of calafatine 2β-*N*-oxide (3) is very close to that of the isomeric oxide (2), and it too incorporates peak *m/z* 222 due to species (4). Once again, therefore, the *N*-oxide group is on the left-hand portion of the dimer as drawn.

Both *N*-oxides are laevorotatory, and their c.d. spectra are closely related, exhibiting a positive first Cotton effect at 245—247 nm, and a negative second Cotton effect at 232—233 nm. Optical properties thus could not be relied upon to establish the stereochemistry at the *N*-oxide centres.

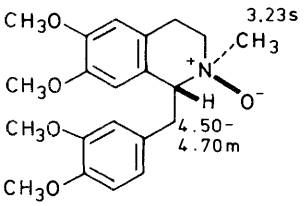
We, therefore, had resort to n.m.r. n.O.e. (nuclear Overhauser effect) difference studies which supplied a clear cut answer to the stereochemical problem.[5] Irradiation of the *N*-methyl singlet at δ 3.26 in calafatine 2α-*N*-oxide (2) produced a 6.1% enhancement of the 1-H multiplet signal at δ 4.20. Conversely, irradiation of the 1-H multiplet led to a 4.4% enhancement of the δ 3.26 *N*-methyl signal. On the other hand, similar irradiation of the δ 3.41 methyl singlet in calafatine 2β-*N*-oxide (3) resulted in no observable n.O.e. for the



(4)



(5)



(6)

δ 4.35 multiplet representing 1-H; while irradiation of the 1-H signal gave no response at the δ 3.41 peak.

It follows that *N*-oxide (2) incorporates a geometry in which the *N*-methyl and 1-H are close to each other, and a *trans*-relationship prevails between the N-2 oxygen and 1-H. Conversely, in *N*-oxide (3), the *N*-2 oxygen and 1-H are *cis* to one another.

The above results should be compared with those reported for the synthetic oxides of laudanosine (5) and (6), whose relative stereochemistry was deduced from simple comparison of n.m.r. chemical shifts.[6] In that instance, it was found that the chemical shifts of the *N*-methyl signals are very close to each other, δ 3.24 for the *trans*-isomer (5) and δ 3.23 for the *cis*-isomer (6). However, in accordance with the results obtained above, the shift for 1-H is further upfield (δ 4.00—4.30) when this hydrogen is *trans* to the oxygen than when it is *cis* (δ 4.50—4.70).

We believe that, at this stage, assignments of configuration for bisbenzylisoquinoline *N*-oxides must still be accompanied by relevant n.m.r. n.O.e. difference studies. This requirement for Overhauser studies may be particularly important when only one *N*-oxide isomer is available, rather than when two are at hand as in the present instance.

It should also be noted in conclusion that in excess of 135 alkaloidal *N*-oxides of all types are known, and that the stereochemistry at the nitrogen centre of a number of these is unknown. The present n.m.r. n.O.e. difference studies approach could prove useful in elucidating some of these problems.[7]

Experimental

General Experimental Procedure.—N.m.r. spectra were obtained in $CDCl_3$ solution using a Bruker 360 MHz instrument. The alkaloid isolation procedure was along classical lines. Methanol extraction of the dried, powdered, whole plant (8 kg), minus the leaves, was followed by column chromatography of the basic extracts over silica gel. Elution was with chloroform containing increasing amounts of methanol. Final purification was by t.l.c. This procedure provided 22 mg of *N*-oxide (2) and 30 mg of *N*-oxide (3).

Calafatine (1): *m/z* 652 (M^+, $C_{39}H_{44}N_2O_7$) (41), 651 (34), 637 (10), 396 (36), 395 (88), 381 (100), 198 (69), 192 (4), and 174 (72); c.d. Δε (nm) (MeOH) 0 (300), +9 (247), 0 (242), −18 (234), 0 (225), +10 (219), and 0 (212); $[\alpha]_D^{25}$ −154° (*c* 0.28, $CHCl_3$).

Calafatine 2α-N-*oxide* (2): $\lambda_{max.}$ (MeOH) 210, 231sh, and 281 nm (log 4.95, 4.67, and 3.91); *m/z* 668 $(M)^+$ (3), 667 (6), 652 (54), 396 (26), 395 (76), 381 (73), 222 (5), 207 (2), 206 (8), 198 (100), 191 (28), and 174 (42); c.d. Δε (nm) (MeOH) 0 (300), +12 (247), 0 (242), −42 (233), 0 (225). +27 (217), and 0 (211); $[\alpha]_D^{25}$ −48° (*c* 0.17, MeOH); t.l.c. R_F 0.26 in the solvent system chloroform–methanol–ammonium hydroxide (90 : 10 : 1) using Merck Silica Gel F-254 glass plates, 0.25 mm thick.

Calafatine 2β-N-*oxide* (3): $\lambda_{max.}$ (MeOH) 206, 229sh, and 280 nm (log 4.91, 4.63, and 3.85); *m/z* 668 $(M)^+$ (2), 667 (1), 652 (44), 396 (28), 395 (80), 381 (76), 222 (3), 206 (6), 198 (100), 191 (8), and 174 (65); c.d. Δε (nm) (MeOH) 0 (300), +15 (245), 0 (240), −38 (232), 0 (224), +25 (217), and 0 (210); $[\alpha]_D^{25}$ −19° (*c* 0.14, MeOH); t.l.c. R_F 0.17 in the same solvent system as above.

Acknowledgements

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