Conformational Analysis of the Novel Antihypertensive Agent Cromakalim (BRL34915)

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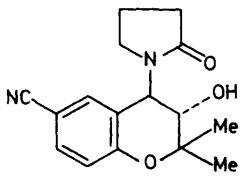
N.m.r. analysis of the novel antihypertensive agent cromakalim shows that it adopts a rigid conformation in solution, similar to that observed in the crystalline state by an *X*-ray crystal structure determination.

Conformational analysis of drug molecules is vital in providing an understanding of their binding to receptors. In this communication we report our studies on the antihypertensive agent cromakalim (**1**) which lowers blood pressure *via* a novel mechanism of action involving activation of potassium channels in vascular smooth muscle.[1,2]

X-Ray crystal structure analysis of (**1**)† showed the conformation displayed in Figure 1 where the pyrrolidone ring is orthogonal to the benzopyran ring and with the pyrrolidone carbonyl on the same side of the benzopyran ring as H(4). The same conformation of (**1**) was found in solution in $CDCl_3$ using nuclear Overhauser enhancement (n.O.e.) difference techniques (Figure 2). Irradiation at H(3) produced positive signals for H(5), H(8), H(4), $CH_3(eq)$, and H(14). Irradiation at H(4) produced positive signals for H(5), H(3), and $CH_3(ax.)$. No n.O.e. was observed between H(4) and H(14) or H(14'). Irradiation at H(5) produced positive signals for H(4) and H(14'). Therefore, (**1**) adopts a rigid conformation in solution similar to that found in the crystalline state. Confirmation that the pyrrolidone ring is not rotating about the C(4)–N(1) bond was obtained from a study of ^{13}C relaxation times[3] (Table 1). This showed that the pyrrolidone carbons are not significantly more mobile than the carbons of the benzopyran nucleus. Furthermore, a variable temperature



Cromakalim (**1**)

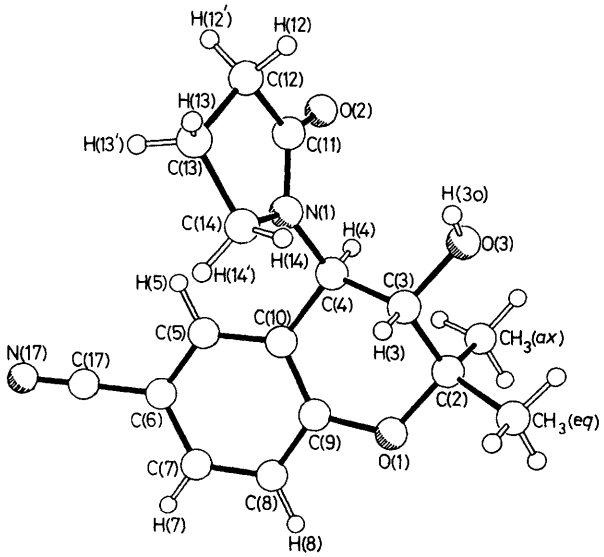


Figure 1. The structure of (**1**), giving atom numbering. The C(3)–C(4)–N(1)–C(14) and C(10)–C(4)–N(1)–C(14) torsion angles are −60 and 63° respectively. There is an intermolecular hydrogen bond between the hydroxy oxygen O(3) and the C(11) carbonyl oxygen O(2); O(3) · · · O(2') 2.71, O(3)H · · · O(2') 1.73 Å, O–H · · · O angle 176°.

† *Crystal data* for (**1**): $C_{16}H_{18}N_2O_3.0.5(CH_3OH)$, M = 302.4, monoclinic, a = 11.037(1), b = 7.683(1), c = 18.887(2) Å, β = 90.81(1)°, U = 1601 Å^3, space group $P2_1/c$, Z = 4, D_c = 1.25 g cm^{-3}, μ(Cu-K_α) = 7 cm^{-1}. The structure was solved by direct methods and refined anisotropically to give R = 0.048, R_w = 0.054 for 1649 independent observed reflections [$|F_o| \geq 3\sigma(|F_o|)$, $\theta \leq 50°$]. Data were measured on a Nicolet R3m diffractometer with Cu-K_α radiation (graphite monochromator) using ω-scans. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

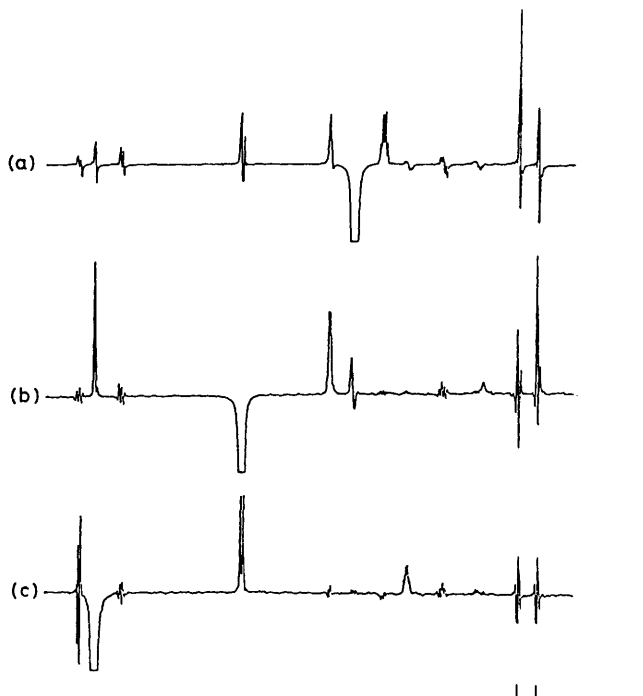


Figure 2. N.O.e. difference experiments with cromakalim. (a) Irradiation at H(3); (b) Irradiation at H(4); (c) Irradiation at H(5); (d) 270 MHz ^1H n.m.r. spectrum, $CDCl_3$. Assignments are based on chemical shift and COSY 45 data.

Table 1. ^{13}C Relaxation times for (**1**).

Carbon	T_1/ms	nT_1/ms[a]
3	510	510
4	490	490
5	460	460
7	390	390
8	480	480
12	320	640
13	380	760
14	290	580
$CH_3(ax)$	490	1470
$CH_3(eq)$	450	1350

[a] nT_1 is the product of T_1 and the number (n) of attached hydrogens on each carbon.

n.m.r. study failed to show any spectral changes between −90 and 150 °C.

The barrier to rotation of C(4)–N(1) was investigated by using the semi-empirical AM1 method[4] in the AMPAC program.[5] Twelve structures with 30° increments in the C(4)–N(1) torsion were used. All internal co-ordinates were optimised except the dihedral specifying the C(4)–N(1) torsion for each structure.

The most stable structure corresponded to that determined by *X*-ray crystallography, although another minimum on the rotation co-ordinate was found at 210° from the *X*-ray structure. This is higher in energy by 2.4 kcal/mol (cal = 4.184 J). If we assume that a Boltzmann distribution of energies exists for this system, then the lower energy minimum would predominate (>98%). However, as the energy difference between the two minima is relatively small, either minimum could be close to the conformation adopted by cromakalim at its receptor.

Received, 31st October 1988; Com. 8/04328K

References

1 T. C. Hamilton, S. W. Weir, and A. H. Weston, *Br. J. Pharmacol.*, 1986, **88**, 103.
2 V. A. Ashwood, R. E. Buckingham, F. Cassidy, J. M. Evans, E. A. Faruk, T. C. Hamilton, D. J. Nash, G. Stemp, and K. Willocks, *J. Med. Chem.*, 1986, **29**, 2194.
3 'Carbon-13 Nuclear Magnetic Resonance Spectroscopy,' eds. G. C. Levy, R. L. Lichter, and G. L. Nelson, 2nd edn., Wiley–Interscience, New York, 1980.
4 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.
5 J. J. P. Stewart, *QCPE Bulletin*, 1986, **6**, 24a.