Tetrahedron, 1964, Vol. 20, pp. 1449 to 1453. Pergamon Press Ltd. Printed in Northern Ireland

THE STRUCTURE OF OXYCOLCHICINE

G. L. BUCHANAN, A. MCKILLOP, A. L. PORTE and J. K. SUTHERLAND
Chemistry Department, University of Glasgow, Scotland

(*Received* 11 *March* 1964)

Abstract—On the basis of chemical and spectroscopic evidence, oxycolchicine has been shown to be a 1:4-oxide (V) of colchicine.

COLCHICINE (I), the principal alkaloid of the Autumn Crocus, undergoes two reactions which are unique in tropolone chemistry, in that the tropolone ring suffers oxidative attack without the loss of a carbon atom. One of these processes has been shown[1] to yield a lactonic acid; the other gives rise to oxycolchicine, and the structural evidence concerning this novel product, which has already been disclosed in two brief communications,[2,3] is now presented in full.

During the early exploratory investigations of the alkaloid colchicine, it was found[4] that oxidation with chromic acid afforded a neutral product, oxycolchicine ($C_{22}H_{23-25}O_7N$), which still contained the N-Ac and the four OMe groups present in colchicine, but which, unlike its precursor, formed a crystalline semicarbazone.[5,6] Although at the time neither the precise formula nor the nature of the oxidation was understood, the appearance of ketonic properties was construed in terms of the H_{23} formula as $>CH_2 \rightarrow >C{=}O$.[4,5] The oxidation reaction was held to support the structure (III) then accepted for colchicine, by establishing the presence of an unsubstituted methylene group,[5] and oxycolchicine was formulated as IV. Much later, when the correct structure of colchicine was appreciated, the problem was reinvestigated by Santary[7] who showed firstly that ring B was unlikely to be attacked under the reaction conditions, and, secondly, that on the basis of the UV absorption spectrum, oxycolchicine no longer possessed the tropolone chromophore. Oxidation was therefore presumed to have damaged ring C.

This has now been corroborated by our own observation that oxycolchicine is reduced to colchiceine (II) by potassium iodide in acetic acid. The regeneration of the tropolone ring under such mild conditions, coupled with the absence of OH absorption in the IR spectrum of oxycolchicine suggests that the "extra" oxygen is located as an ether bridge in ring C. The ketonic properties of oxycolchicine thus arise from the carbonyl function of colchicine, unmasked when the tropolone conjugation is interrupted, and oxycolchicine is properly formulated as $C_{22}H_{25}O_7N$. Its IR spectrum ($CHCl_3$) shows NH (3443 cm^{-1}) and C=O (1700, 1683 cm^{-1}) bands;

[1] K. Ahmad, G. L. Buchanan and J. W. Cook, *J. Chem. Soc.* 3278 (1957).
[2] G. L. Buchanan and J. K. Sutherland, *Chem. & Ind.* 418 (1958).
[3] G. L. Buchanan, A. L. Porte and J. K. Sutherland, *Chem. & Ind.* 859 (1962).
[4] S. Zeisel and A. Friedrich, *Monatsh.* **34**, 1181 (1913).
[5] A. Windaus, *Liebs. Ann.* **439**, 59 (1924).
[6] Oxycolchicine gives an amorphous oxime[4] and an intractable 2,4-dinitrophenylhydrazone, but no other crystalline carbonyl derivative has been prepared.
[7] F. Santary, *Chem. Listy* **46**, 488 (1952).

the latter are assigned respectively to ketone and amide functions, and this has been verified by borohydride reduction to dihydro-oxycolchicine, which shows only amide absorption.[8] The dihydro derivative is readily transformed into colchiceine (II) by treatment with mineral acid, thus achieving stepwise, the regeneration of the tropolone ring described above. Contrary to earlier reports,[5] oxycolchicine absorbs only three molecular equivalents of hydrogen on catalytic reduction, and the product, hexahydro-oxycolchicine, forms a *mono*acetate whose IR spectrum reveals no unesterified hydroxyl function. The ether bridge had therefore survived reduction and, on this evidence, a 1:2-oxide structure seems unlikely. Lithium aluminium hydride reduces oxycolchicine to a tetrahydrodeoxy derivative which consumes 2 molecular equivalents of hydrogen on reduction, confirming the degree of unsaturation in ring C. Oxycolchicine is thus a doubly unsaturated oxido-ketone, and the distribution of these groupings within ring C is further circumscribed by our observation that oxycolchicine is unaffected by ethereal perphthalic acid.

The UV absorption spectrum of oxycolchicine also provides useful structural information. It has already been noted (Table 1) that olefinic conjugation with the trimethoxy benzene ring gives rise to a typical UV absorption curve. Non-conjugated reference compounds similarly show a typical, but significantly different pattern (Table 1) and it is obvious by inspection that oxycolchicine is of the latter type. The

TABLE 1. ULTRAVIOLET SPECTRA

	λ_{max}^{EtOH} (log ε)	λ_{min}^{EtOH} (log ε)
Oxycolchicine	281 mμ (2·51)	264 mμ (2·43)
Dihydro-oxycolchicine	273 mμ (2·95)	260 mμ (2·85)
Hexahydro-oxycolchicine	274 mμ (2·85)	254 mμ (2·5)
Non-conjugated derivatives[9]	274–280 mμ (2·95–3·18)	252–262 mμ (2·45–2·9)
Conjugated derivatives[10]	254–258 mμ (4·1)	241–245 mμ (3·95)

location of the double bonds as in V now follows unequivocally from the NMR spectrum (Fig. 1) which shows a one proton singlet at 4·7 τ [$H_{(h)}$] and an AB quartet (two protons) at 2·54 and 3·99 τ assigned to $H_{(f)}$ and $H_{(a)}$ respectively [$J_{H_{(a)}-H_{(f)}}$ 5·4 c/s]; the downfield displacement of the $H_{(f)}$ signal and the reduction in magnitude of $J_{H_{(a)}-H_{(f)}}$ are both due to the effect of the adjacent oxygens. Dihydro- and hexahydro-oxycolchicine are therefore formulated at VI and VII, and the lithium aluminium hydride reduction product as VIII.

[8] This reduction also provides additional evidence of the ketonic character of oxycolchicine. The semicarbazone, which is frequently quoted to prove this point, has never been satisfactorily analysed for any element other than nitrogen.[5] (Experimental.)

[9] Data collected from the epoxides described by A. Rapoport, A. R. Williams, J. E. Campion and D. E. Pack, in *J. Amer. Chem. Soc.* **76**, 3693 (1954); H. Rapoport (private communication) and from compounds described by G. L. Buchanan and J. K. Sutherland in *J. Chem. Soc.* 2334 (1957); J. K. Sutherland, *Ph.D. thesis*, Glasgow University (1957).

[10] H. Rapoport, J. E. Campion and J. E. Gordon, *J. Amer. Chem. Soc.* **77**, 2389 (1955); H. J. E. Loewenthal, *J. Chem. Soc.* 1421 (1961).

The expression (V), which has recently been verified by F. Santary,[11] satisfactorily accommodates the foregoing data, but a few points deserve special comment. The low reactivity of the unconjugated olefinic double bond toward perphthalic acid is consistent with its occurrence in an α-oxygenated dihydrofuran system.[12] Equally remarkable is the high ν_{CO} (1700 cm^{-1}) of oxycolchicine, but there is some evidence that α-alkoxy substituents cause a high frequency shift in the ν_{CO} of cyclic ketones,[13] and in an analogous molecule (IX), the ν_{CO} is found[14] to be 1713 cm^{-1}. The conversion of colchicine to a 1:4-oxide (V) by chromic acid oxidation recalls the formation of 1:2-oxides from olefins under similar conditions[15] and can be rationalized as a transannular modification (X → V) of the same mechanism;[16] the product presumably survives further attack because of its insolubility. The mechanism of regeneration of the tropolone ring has already been discussed.[3]

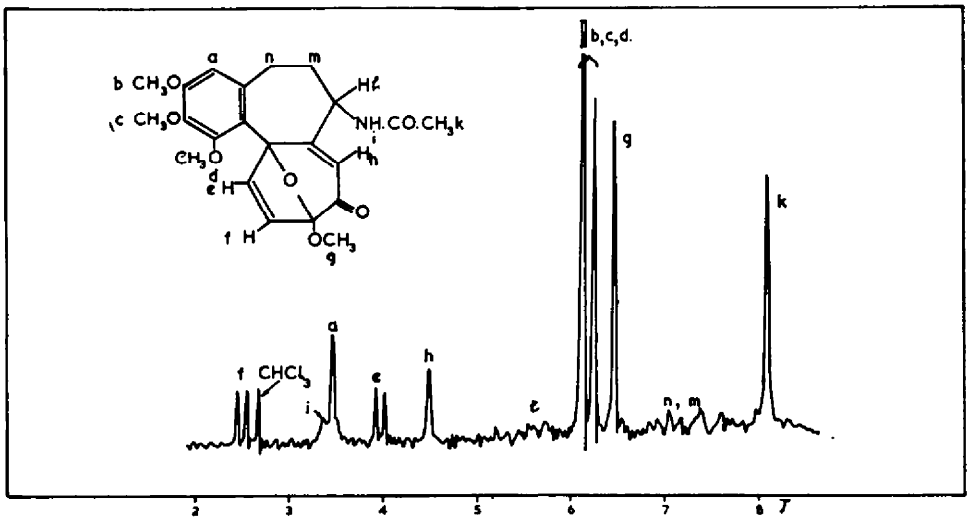


FIG. 1. NMR Spectrum of oxycolchicine in $CDCl_3$.

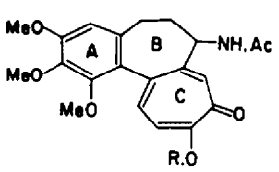
EXPERIMENTAL

UV and IR spectra were measured on Unicam S.P. 500 and S.P. 100 spectrophotometers, respectively, the NMR spectrum was measured in $CDCl_3$ by Mr. J. Gall on a Perkin Elmer 60 m/c instrument: microanalyses were carried out by Mr. J. M. L. Cameron, B.Sc. and his staff.

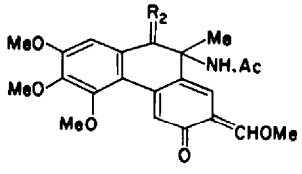
Oxycolchicine. In contrast to the literature[4] method the following modification can be carried out on a 5 g scale in one operation, without loss of yield.

Potassium dichromate (11·4 g) in water (100 ml) and conc. H_2SO_4 (10 ml) was heated to ca. 90°, and treated with a solution of colchicine (5 g) in water (50 ml) preheated to ca. 60°. The solution darkened and frothed, depositing crystalline oxycolchicine. The mixture was swirled from time to time and, after 1 hr, it was reheated to boiling and set aside to cool. The product was filtered off,

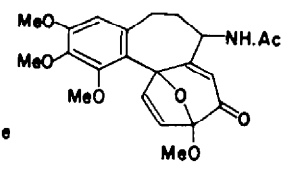
[11] A. D. Cross, F. Santary and B. Trivedi, *Coll. Czech. Chem. Comm.* **88**, 3402 (1963).
[12] J. C. Sheehan and B. M. Bloom, *J. Amer. Chem. Soc.* **74**, 3825 (1952); cf. also B. Belleau and Yum-Kin Au-Young, *Ibid*, **85**, 64 (1963).
[13] L. F. Fieser, T. Goto and B. K. Bhattacharrya, *J. Amer. Chem. Soc.* **82**, 1700 (1960).
[14] G. L. Buchanan and D. B. Jhaveri, *J. Org. Chem.*, **26**, 4295 (1961).
[15] W. J. Hickinbottom, D. Peters and D. G. M. Wood, *J. Chem. Soc.* 1360 (1955).
[16] W. A. Waters, *Quart. Rev.* **12**, 287 (1958).



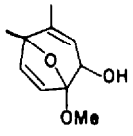
I; R = Me
II; R = H



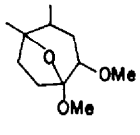
III; R_2 = H_2
IV; R_2 = O



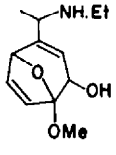
V



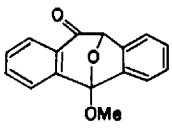
VI



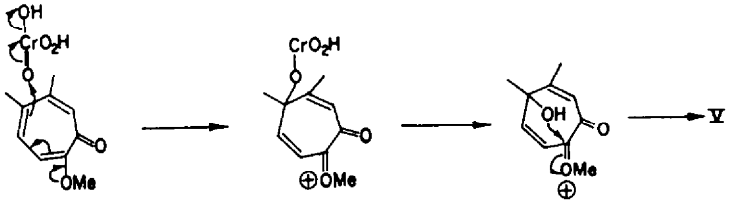
VII



VIII



IX



X

washed with water, dried and recrystallized from aqueous ethanol, m.p. 265–266°, yield 1·4–1·6 g. Oxycolchicine consumed no appreciable amount of perphthalic acid on standing 24 hr in ethereal solution.

The semicarbazone, prepared as described,[5] crystallized from aqueous methanol, m.p. 222–224°. (Found: C, 53·30 53·2; H, 6·4, 6·6; N, 11·7; MeO, 24·8%) λ_{max}^{EtOH} 295 mμ (ε 19,000).

Conversion to colchiceine. Oxycolchicine (50 mg), KI (200 mg) and acetic acid (15 ml) were heated on the steam bath for 5 hr during which time iodine was liberated. After standing overnight, the solution was shaken in the presence of H_2 and Adams PtO_2 catalyst until the colour was discharged. The mixture was filtered and the filtrate was concentrated *in vacuo*, yielding a solid which was extracted with ethyl acetate. Evaporation of the extract afforded an oily product (29 mg) which was charcoaled in ethanolic solution and crystallized from water, yielding colchiceine, m.p. and mixed m.p. 139–140°. Its IR and UV spectra were identical with those of authentic material. The same product is formed on refluxing oxycolchicine with Zn dust in acetic acid.

Dihydro-oxycolchicine. Oxycolchicine (100 mg) in methanol (3 ml) was treated with KBH_4 (100 mg) in water (3 ml) and left at room temp for 24 hr. The crystalline product was washed with methanol, m.p. 275–280° (dec.) Attempted recrystallization led to decomposition, and a sample prepared under analytical conditions analysed as follows. (Found: C, 63·4; H, 6·7; N, 3·5. $C_{22}H_{27}O_7N$ requires: C, 63·3; H, 6·5; N, 3·35%), ν_{CO}^{nujol} 1660 cm^{-1}.

Dihydro-oxycolchicine (20 mg) and dil. HCl aq (1·5 ml, 0·05N) was heated on the steam bath for 35 min, filtered and allowed to stand, when colchiceine, m.p. and mixed m.p.140–141°, crystallized out.

Hexahydro-oxycolchicine. Oxycolchicine (260 mg) in acetic acid (10 ml) was shaken in presence of H_2 with Adams PtO_2 catalyst (150 mg). Three mole equiv were absorbed in 4 hr; the solution was filtered and concentrated *in vacuo*, affording the hexahydride which crystallized from ethanol in colourless needles, m.p. 271–275°. (Found: C, 62·2; H, 7·3; N, 3·4; CH_3CO, 7·6. $C_{22}H_{31}O_7N$ requires: C, 62·7; H, 7·4; N, 3·3; CH_3CO, 10·2%).

The IR spectrum ($CHCl_3$) showed OH (3539 cm^{-1}; $\Delta\nu_{\frac{1}{2}}$ 48 cm^{-1}; ε, 70), NH (3436 cm^{-1}; $\Delta\nu_{\frac{1}{2}}$ 19 cm^{-1}; ε, 115), and amide C=O (1666 cm^{-1} $\Delta\nu_{\frac{1}{2}}$ 25 cm^{-1}; ε, 550) bands.

Acetyl derivative. The hexahydride (64 mg) in acetic anhydride (2 ml) and pyridine (3 ml) was

left 48 hr at room temp and then concentrated *in vacuo*. The crude product was crystallized from methanol in colourless needles, m.p. 268–270°. (Found: C, 61·8; H, 6·8; N, 3·4; CH_3CO, 15·9; MeO, 24·9. $C_{24}H_{33}O_8N$ requires: C, 62·25; H, 7·2; N, 3·0; $2CH_3CO$, 18·5; 4MeO, 24·55%). The IR spectrum in $CHCl_3$ shows NH (3435 cm^{-1}; $\Delta\nu\frac{1}{2}$ 18 cm^{-1}; ε, 104), amide CO (1667 cm^{-1}: $\Delta\nu\frac{1}{2}$ 26 cm^{-1}; ε, 560) and ester CO (1734 cm^{-1}; $\Delta\nu\frac{1}{2}$ 31 cm^{-1}; ε, 400) bands.

Lithium aluminium hydride reduction. Oxycolchicine (500 mg) in a Soxhlet thimble, was extracted 3 days with ether containing $LiAlH_4$ (1 g). After decomposing the excess hydride with ethyl acetate, sat. $(NH_4)_2SO_4$ aq was added and the suspension was thoroughly extracted with ether. Evaporation yielded a gummy solid (183 mg) which was triturated with ether and crystallized from ethanol, affording the hydroxyamine, m.p. 236–238°. (Found: C, 65·05; H, 6·9; N, 3·5. $C_{22}H_{29}O_6N$ requires: C, 65·5; H, 7·2; N, 3·5%). The product was soluble in dil. mineral acid and showed ν_{max}^{nujol} 3240 (NH), 3280–2980 (OH), 1596 (Ar) cm^{-1}.

On catalytic reduction in acetic acid in presence of Adams PtO_2 catalyst one mole equiv. H_2 was absorbed rapidly and a second more slowly.

Acknowledgements—The authors acknowledge their indebtedness to Dr. Glenn E. Ullyot of Smith, Kline and French Laboratories, Philadelphia, U.S.A., for a generous gift of colchicine. Two of us A. McK. and J. K. S.) gratefully acknowledge grants from D.S.I.R.