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Semisyntheses, X-Ray Crystal Structures and Tubulin-Binding Properties of 7-Oxodeacetamidocolchicine and 7-Oxodeacetamidoisocolchicine

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Abstract

Commercially available (−)-colchicine (1) has been converted, via deacetylcolchiceine (4), into a mixture of 7-oxodeacetamidocolchicine (2) and 7-oxodeacetamidoisocolchicine (3). The X-ray structures and tubulin-binding properties of these title ketones are described.

Introduction

As part of an on-going program within these laboratories to develop efficient synthetic routes to the antimitotic agent (−)-colchicine (1)[1,2] and its congeners,[3–5] we required preparatively useful quantities of the ketone (2). This latter compound is of interest because it might be expected that reductive amination of the C 7 carbonyl moiety could be achieved with some level of enantiocontrol thereby allowing the synthesis of the naturally occurring antipode of the alkaloid (1). Such an objective is important since only (−)-colchicine appears to display a useful range of biological properties.[6] While we have recently realized a fully regiocontrolled total synthesis of ketone (2),[7] there are still logistical problems associated with trying to use this route to accumulate sufficient quantities of material for the enantioselective reduction studies. Consequently, we now describe a reliable procedure for the degradation of commercially available (−)-colchicine to a (separable) mixture of the required ketone and regioisomer (3). In view of

[1] Banwell, M. G., Lambert, J. N., Corbett, M., Greenwood, R. J., Gulbis, J. M., and Mackay, M. F., *J. Chem. Soc., Perkin Trans. 1*, 1992, 1415.
[2] Banwell, M. G., Lambert, J. N., Mackay, M. F., and Greenwood, R. J., *J. Chem. Soc., Chem. Commun.*, 1992, 974.
[3] Banwell, M. G., Herbert, K. A., Buckleton, J. R., Clark, G. R., Rickard, C. E. F., Lin, C. M., and Hamel, E., *J. Org. Chem.*, 1988, **53**, 4945.
[4] Banwell, M. G., Gravatt, G. L., Buckleton, J. S., Clark, G. R., and Rickard, C. E. F., *J. Chem. Soc., Chem. Commun.*, 1989, 865.
[5] Banwell, M. G., Cameron, J. M., Collis, M. P., Crisp, G. T., Gable, R. W., Hamel, E., Lambert, J. N., Mackay, M. F., Reum, M. E., and Scoble, J. A., *Aust. J. Chem.*, 1991, **44**, 705.
[6] Brossi, A., Yeh, H. J. C., Chrzanowska, M., Wolff, J., Hamel, E., Lin, C. M., Quin, F., Suffness, M., and Silverton, J., *Med. Res. Rev.*, 1988, **8**, 77, and references therein.
[7] Lambert, J. N., Ph.D. Thesis, University of Melbourne, 1992.

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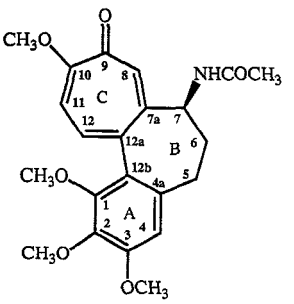
our continued interest in structure/activity relationships within the colchicinoid series,[3–5] we also report on the X-ray structures and tubulin-binding properties of the title ketones. Tubulin-binding activity is frequently used as a relatively simple prescreen to identify those colchicinoids more likely to possess useful *in vivo* antitumour activity.[6]



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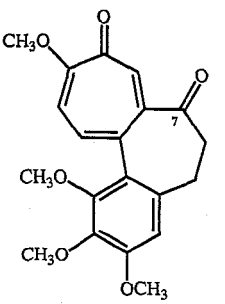

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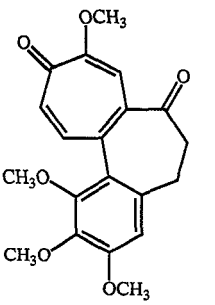

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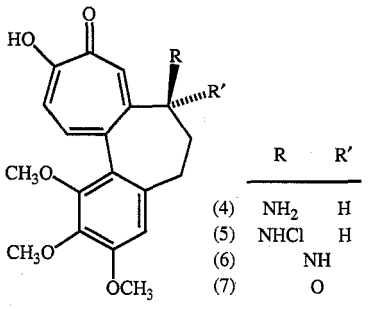
Results and Discussion

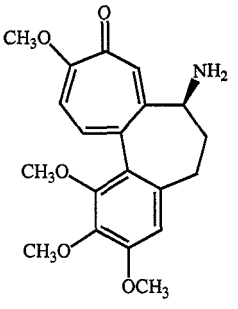
Synthetic Studies

While at least two catabolic processes which degrade (–)-colchicine (1) to 7-oxodeacetamidocolchicine (2) have been identified,[8,9] the work described herein has focused on using chemical means for effecting this conversion. In the first instance, attempts were made to reproduce a procedure described[10] in the French patent literature. To these ends, commercially available (1) was treated with aqueous sulfuric acid at 100° and, after workup, deacetylcolchiceine (4)[11] was obtained in 70% yield. Reaction of (4) with *N*-chlorosuccinimide resulted in chlorine atom transfer and formation of (5) (58%). The physical data obtained for this latter compound matched those reported[10] and the spectral data were fully consistent with the assigned structure. However, when compound (5) was treated under the specified[10] conditions with methanolic potassium hydroxide, only small amounts (<25%) of the elimination product (6) could be obtained. The use of alternative bases, e.g. 1,8-diazabicyclo[5.4.0]undec-7-ene (dbu), failed to change this outcome. Evidence for the formation of the unstable imine (6) in these reactions rested on isolation of the known[12] ketone (7) after the crude material had been subjected to acid-catalysed hydrolysis. While *O*-methylation of (7) (with diazomethane)[13] gave a 1 : 1·2 mixture of (2) and (3) which could be separated chromatographically, the low overall yield associated with this reaction sequence prompted an examination of alternative routes to (2).

[8] Zeitler, H.-J., and Niemer, H., *Hoppe-Seyler's Z. Physiol. Chem.*, 1969, **350**, 366.
[9] Al-Tel, T. H., Abu Zarga, M. H., Sabri, S. S., Freyer, A. J., and Shamma, M., *J. Nat. Prod.*, 1990, **53**, 623.
[10] French Pat. 1,375,049 to Roussel-UCLAF (1963).
[11] Raffauf, R. F., Farren, A. L., and Ullyot, G. E., *J. Am. Chem. Soc.*, 1953, **75**, 5292.
[12] Iorio, M. A., Brossi, A., and Silverton, J. V., *Helv. Chim. Acta*, 1978, **61**, 1213.
[13] Banwell, M. G., Lambert, J. N., Reum, M. E., and Onrust, R., *Org. Prep. Proc. Int.*, 1988, **20**, 393.



	R	R′
(4)	NH_2	H
(5)	NHCl	H
(6)	NH	
(7)	O	



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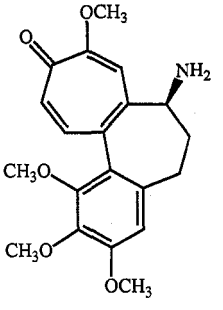
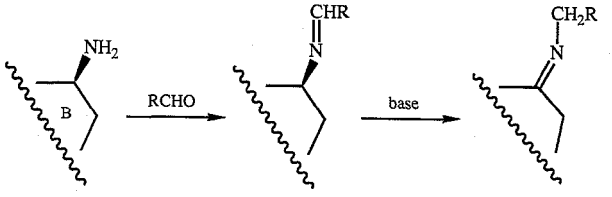


(9)

Brossi and coworkers have described[12] the application of a biomimetic oxidation sequence (Scheme 1) for the conversion of (4) into ketone (7). Thus, reaction of the Schiff base [cf. (11)] derived from (4) [cf. (10)] and benzaldehyde with potassium hydroxide resulted in partial double-bond migration and formation of 7-benzyliminodeacetamidocolchiceine [cf. (12)]. Acid-catalysed hydrolysis of the latter compound then afforded the 7-oxo compound (7), albeit in modest overall yield.



(10) (11) (12) Scheme 1

Since the publication of Brossi's procedure, two groups have reported on the utility of isonicotinaldehyde (pyridine-4-carbaldehyde)[14] and 4-formyl-1-methylpyridinium benzenesulfonate[15] as reagents for effecting the conversion of amines to carbonyl compounds under mild conditions. Consequently, we examined the applicability of such compounds to the oxidation process shown in Scheme 1. Attempts to implement such a sequence by using amine (4) and isonicotinaldehyde under the specified conditions[14] failed to give the desired product (7). When the regioisomeric tropolone *O*-methyl ethers (8) and (9), obtained by treating (4) with diazomethane, were reacted (either as a mixture or separately) with the same aldehyde, low yields (0–20%) of the ketones (2) and (3) were obtained. In contrast, sequential treatment of the mixture of (8) and (9) with 4-formyl-1-methylpyridinium *p*-toluenesulfonate,† dbu and aqueous oxalic acid afforded the corresponding oxidation products (2) and (3) which could be separated by

† 4-Formyl-1-methylpyridinium *p*-toluenesulfonate, rather than 4-formyl-1-methylpyridinium benzenesulfonate, was used in this reaction sequence simply because of the ready availability of methyl *p*-toluenesulfonate within these laboratories.

[14] Ohta, S., and Okamoto, M., *Synthesis*, 1982, 756.
[15] Buckley, T. F., and Rapoport, H., *J. Am. Chem. Soc.*, 1982, **104**, 4446.

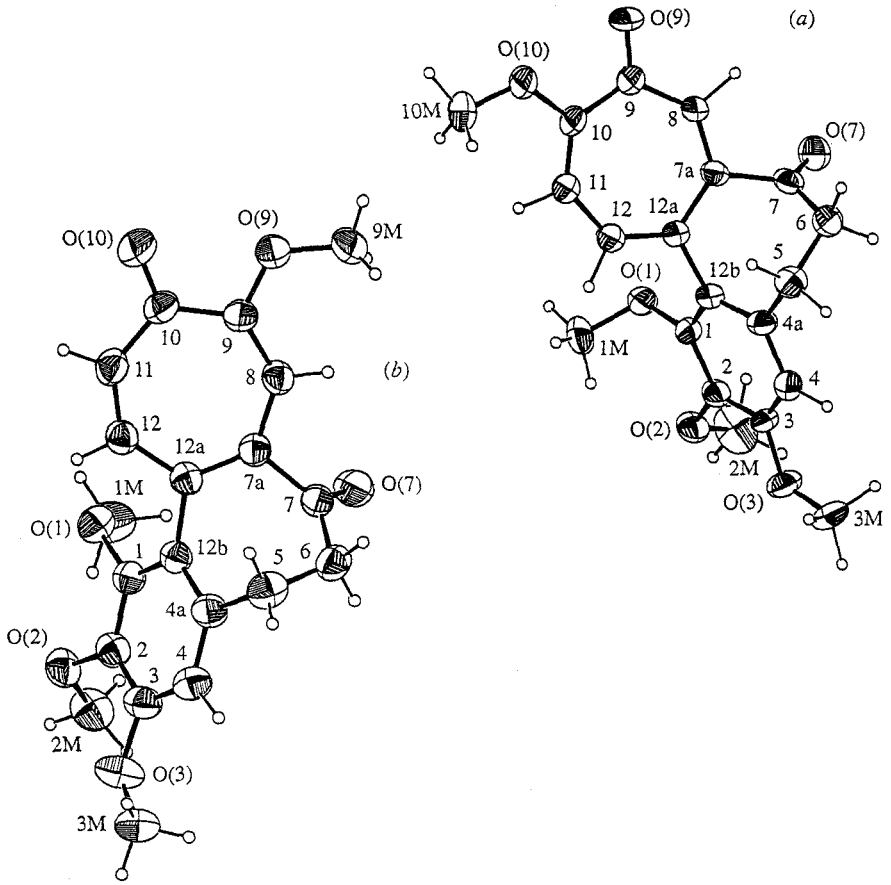


Fig. 1. Perspective view of (*a*) compound (2); (*b*) compound (3) with thermal ellipsoids scaled to 50% probability. Hydrogen atoms are represented by spheres of arbitrary radius. For carbon atoms the C symbol is omitted.

Table 1. Selected torsional angles (degrees) for compounds (2) and (3)

E.s.d. values are given in parentheses

Atoms	(2)	(3)
C(1M)–O(1)–C(1)–C(2)	60·9(6)	−64·1(5)
C(2M)–O(2)–C(2)–C(3)	−75·1(6)	−69·3(5)
C(3M)–O(3)–C(3)–C(4)	3·0(7)	12·3(5)
C(1)–C(12b)–C(12a)–C(12)	60·9(6)	52·3(5)
C(12a)–C(7a)–C(7)–C(6)	−67·1(6)	−67·1(5)
C(7a)–C(7)–C(6)–C(5)	36·6(6)	27·7(6)
C(7)–C(6)–C(5)–C(4a)	49·9(5)	56·5(5)
C(6)–C(5)–C(4a)–C(12b)	−68·1(5)	−67·7(7)
C(5)–C(4a)–C(12b)–C(12a)	−10·7(7)	−11·3(6)
C(4a)–C(12b)–C(12a)–C(7a)	61·6(6)	55·2(6)
C(12b)–C(12a)–C(7a)–C(7)	−5·1(6)	3·1(6)
C(8)–C(7a)–C(7)–O(7)	−62·7(6)	−61·8(6)
O(9)–C(9)–C(10)–O(10)	−8·2(6)	−2·1(6)
C(8)–C(9)–O(9)–C(9M)		0·1(6)
C(9)–C(10)–O(10)–C(10M)	−177·8(4)	

medium-pressure liquid chromatography (m.p.l.c.). The overall (isolated) yields of (2) and (3) [from (4)] were 34 and 39%, respectively, and crystals of both compounds suitable for X-ray studies were readily obtained.

X-Ray Crystallographic Studies of Compounds (2) and (3)

The molecular conformations of (2) and (3) are illustrated in Fig. 1 (see also Table 1). The troponoid C-ring atoms are coplanar to within 0·09(1) and 0·02(1) Å in (2) and (3), respectively, with the oxo and methoxy groups lying close to their associated ring plane. As in colchicine[16] and isocolchicine[17] the C-rings exhibit clear alternation of the long and the short bonds (Table 2). The B-rings adopt shallow boat forms with the exocyclic oxo substituent at C(7) twisted by about 62° from the C-ring plane. The relative orientation of the A/C rings is reflected in the torsional angle C(1)–C(12b)–C(12a)–C(12) of 60·9(6) and 52·3(5)° in compounds (2) and (3), respectively, compared with the value of 53° in colchicine and 55° in isocolchicine.

Table 2. Interbond lengths (Å) and angles (degrees) associated with the C-rings in compounds (2) and (3)

E.s.d. values for the bond lengths are given in parentheses, and for the angles range from 0·3 to 0·4°

Atoms	(2)	(3)	Atoms	(2)	(3)
C(7a)–C(8)	1·367(8)	1·425(7)	C(12a)–C(7a)–C(8)	129·7	130·1
C(8)–C(9)	1·452(8)	1·369(5)	C(7a)–C(8)–C(9)	132·8	131·9
C(9)–C(10)	1·460(6)	1·484(6)	C(8)–C(9)–C(10)	122·1	128·1
C(10)–C(11)	1·374(7)	1·455(7)	C(9)–C(10)–C(11)	127·8	121·6
C(11)–C(12)	1·421(8)	1·357(4)	C(10)–C(11)–C(12)	130·7	132·8
C(12)–C(12a)	1·360(7)	1·437(4)	C(11)–C(12)–C(12a)	131·3	131·4
C(12a)–C(7a)	1·434(6)	1·375(7)	C(12)–C(12a)–C(7a)	124·0	123·9
C(10)–O(10)	1·350(7)	1·227(5)	C(8)–C(7a)–C(7)	114·1	112·7
C(9)–O(9)	1·234(7)	1·342(6)	C(7)–C(7a)–C(12a)	116·1	117·0
C(12a)–C(12b)	1·493(8)	1·497(7)	C(8)–C(9)–O(9)	122·1	123·2
			C(10)–C(9)–O(9)	119·6	108·6
			C(9)–C(10)–O(10)	110·2	119·4
			C(11)–C(10)–O(10)	122·0	119·0
			C(12)–C(12a)–C(12b)	118·0	115·7

As is generally observed in solid-state colchicinoid structures containing three methoxy substituents on the A-ring, those at C(1) and C(2) are approximately orthogonal to the ring plane whilst the methoxy at C(3) is approximately coplanar with it. In (2) the methoxy groups at C(1) and C(2) point in the opposite direction whereas in (3) they point in the same direction, this difference no doubt being a consequence of the differing packing modes in the two crystals. The molecules are present in the structures as mirror image conformers. The carbon and oxygen atom coordinates are shown in Tables 3 and 4 for (2) and (3), respectively.

[16] Lessinger, L., and Margulis, T. N., *Acta Crystallogr., Sect. B*, 1978, **34**, 578.
[17] Lessinger, L., and Margulis, T. N., *Acta Crystallogr., Sect. B*, 1978, **34**, 1556.

Tubulin-Binding Studies

Since analogues of colchicine lacking the acetamido side chain at C(7) retain good activity as tubulin-binding agents,[18–21] it was relevant to determine whether the colchicine analogue (2) and the isocolchicine analogue (3) would behave similarly and thereby significantly inhibit tubulin polymerization. These compounds were compared in simultaneous experiments with colchicine (1) and two closely related compounds (13) and (14) which had been evaluated previously under somewhat different reaction conditions.[21] The results are summarized in Table 5.

Table 3. Fractional atomic coordinates and equivalent isotropic temperature factors (Å)^2 of the non-hydrogen atoms for compound (2)

E.s.d. values are given in parentheses. B_{eq} (Å^2) calculated from the refined anisotropic temperature parameters

$$B_{eq} = 8\pi^2 U_{eq} = \tfrac{8}{3}\pi^2 \sum_i \sum_j U_{ij}\, a_i^*\, a_j^*\, a_i . a_j$$

Atom	10^4x	10^4y	10^4z	B_{eq}
C(1)	6742(6)	8029(4)	5196(4)	2·8(2)
C(2)	5845(6)	7877(4)	4441(4)	2·9(2)
C(3)	4291(6)	6996(4)	4921(4)	2·9(2)
C(4)	3693(6)	6199(4)	6134(4)	3·0(2)
C(4a)	4615(6)	6320(4)	6885(4)	2·9(2)
C(5)	4062(6)	5382(4)	8200(4)	3·4(2)
C(6)	5677(6)	4342(4)	8463(6)	3·5(2)
C(7)	7614(7)	4995(4)	7994(4)	3·0(2)
C(7a)	7742(6)	6399(4)	8037(4)	2·6(1)
C(8)	8713(6)	6386(4)	8790(4)	2·9(2)
C(9)	9112(7)	7453(4)	9139(4)	3·6(2)
C(10)	8210(6)	8820(4)	8818(4)	3·1(2)
C(11)	7153(6)	9382(4)	8027(4)	3·4(2)
C(12)	6660(6)	8833(4)	7303(4)	3·0(2)
C(12a)	6882(6)	7540(4)	7273(4)	2·7(1)
C(12b)	6096(6)	7275(4)	6437(4)	2·7(2)
C(1M)	8124(7)	10312(4)	4090(4)	4·4(2)
C(2M)	7477(8)	7920(5)	2513(4)	5·9(2)
C(3M)	1749(7)	6190(5)	4581(5)	4·5(2)
C(10M)	7785(8)	10887(4)	9259(5)	5·1(2)
O(1)	8315(4)	8850(3)	4766(3)	3·2(1)
O(2)	6476(4)	8659(3)	3228(3)	3·6(1)
O(3)	3424(4)	7009(3)	4115(3)	3·9(1)
O(7)	9046(5)	4402(3)	7637(3)	4·2(1)
O(9)	10222(6)	7174(3)	9745(3)	6·1(2)
O(10)	8584(6)	9531(3)	9403(3)	4·2(1)

[18] Hamel, E., in 'Microtubule Proteins' (Ed. J. Avila) pp. 89–191 (CRC Press: Boca Raton 1990).
[19] Bhattacharyya, B., Howard, R., Maity, S. N., Brossi, A., Sharma, P. N., and Wolff, J., *Proc. Natl Acad. Sci. U.S.A.*, 1986, **83**, 2052.
[20] Banerjee, A., Barnes, L.D., and Luduena, R. F., *Biochim. Biophys. Acta*, 1987, **913**, 138.
[21] Boye, O., Itoh, Y., Brossi, A., and Hamel, E., *Helv. Chim. Acta*, 1989, **72**, 1690.

In terms of an IC_{50} value for inhibition of the extent of polymerization, the ketone (2) (IC_{50} 1·1 μM) was nearly twice as active as colchicine (IC_{50} 1·9 μM). In contrast, the isocolchicine ketone (3) had little inhibitory effect on the reaction (IC_{50} >40 μM). Deacetamidocolchicine (13), lacking the C7 substituent, was nearly identical, within experimental error, to colchicine in its inhibitory activity, while the C5–C6 dehydro congener (14) (IC_{50} 0·98 μM) was even more active than ketone (2). It would appear that the conformational constraints imposed

Table 4. Fractional atomic coordinates and equivalent isotropic temperature factors (Å)^2 of the non-hydrogen atoms for compound (3)

E.s.d. values are given in parentheses. B_{eq} (Å^2) calculated from the refined anisotropic temperature parameters

$$B_{eq} = 8\pi^2 U_{eq} = \tfrac{8}{3}\pi^2 \sum_i \sum_j U_{ij}\, a_i^*\, a_j^*\, \boldsymbol{a}_i \,.\, \boldsymbol{a}_j$$

Atom	10^4x	10^4y	10^4z	B_{eq}
C(1)	4725(3)	9689(3)	5916(4)	3·02(6)
C(2)	5600(3)	9539(3)	6276(4)	3·38(6)
C(3)	5929(3)	8355(3)	6037(4)	3·73(7)
C(4)	5394(3)	7291(3)	5534(4)	3·77(7)
C(4a)	4509(3)	7429(3)	5186(4)	3·40(7)
C(5)	3929(3)	6267(3)	4733(5)	4·05(8)
C(6)	3581(3)	5885(3)	5794(5)	3·93(8)
C(7)	3124(3)	6982(3)	6187(4)	3·37(7)
C(7a)	2696(3)	8049(3)	5191(4)	2·94(6)
C(8)	1793(3)	8076(3)	4845(4)	3·14(6)
C(9)	1173(3)	8853(3)	3996(4)	3·23(6)
C(10)	1280(3)	9964(3)	3192(4)	3·58(7)
C(11)	2120(3)	10386(3)	3247(4)	3·55(7)
C(12)	2921[A]	9949(3)	3931[A]	3·21(6)
C(12a)	3221(3)	8865(3)	4820(4)	2·95(6)
C(12b)	4166(3)	8648(3)	5335(4)	2·96(6)
C(1M)	4578(3)	11389(4)	7340(5)	5·43(10)
C(2M)	6702(3)	10454(4)	8101(5)	4·82(9)
C(3M)	7143(4)	7243(5)	5881(6)	5·66(12)
C(9M)	75(3)	7731(4)	4489(5)	4·41(9)
O(1)	4374(2)	10882(2)	6047(3)	3·75(5)
O(2)	6126(2)	10606(2)	6761(4)	4·00(5)
O(3)	6797(2)	8338(3)	6342(4)	5·12(7)
O(7)	3065(3)	7026(3)	7253(4)	4·65(6)
O(9)	340(2)	8722(2)	3794(4)	4·02(5)
O(10)	647(2)	10549(3)	2470(4)	5·83(7)

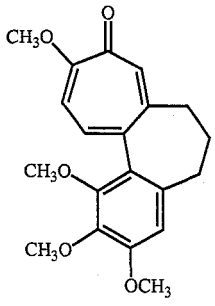
[A] Atom coordinates used to define origin.

Table 5. Effects of ketones (2) and (3) and related compounds on tubulin polymerization

Reaction conditions for determining inhibitory effects are described in detail in the text; s.d. denotes standard deviation

Compound	(1)	(2)	(3)	(13)	(14)
Inhibition of tubulin polymerization, IC_{50}±s.d. (μM)	1·9±0·2	1·1±0·02	>40	1·5±0·1	0·98±0·2

by converting any one of C(5)–C(7) into an sp^2-centre enhances the interaction of a colchicinoid with tubulin. With allocolchicinoids, however, this type of modification has little effect on activity.[21]



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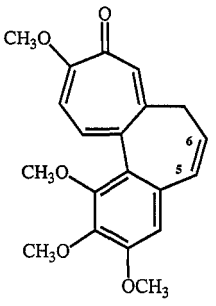

(14)

Experimental

Radial chromatography was performed on a Chromatotron (Harrison Research, Palo Alto/TC Research, Norwich) by using silica gel 60 PF_{254} (Merck). Petroleum spirit refers to the fraction with b.p. 40–60°. Other general experimental procedures have been reported elsewhere.[22] 4-Formyl-1-methylpyridinium *p*-toluenesulfonate was prepared by the procedure reported[15] for the synthesis of 4-formyl-1-methylpyridinium benzenesulfonate with the exception that methyl *p*-toluenesulfonate was used instead of methyl benzenesulfonate.

Deacetylcolchiceine (4)

A mixture of (–)-colchicine (Aldrich) (1·00 g, 2·5 mmol) and sulfuric acid (100 ml of a 20% aqueous solution) was heated at 100° for 6 h. After cooling, solid sodium carbonate was added in small portions to the magnetically stirred reaction mixture until a light yellow frothy mass had formed and the pH of the liquid phase was 7·5. The solid was isolated by vacuum filtration and immediately recrystallized (methanol/water, 95:5) to afford the crude product (0·98 g). A second recrystallization (methanol/water) and drying (60°/0·5 mm Hg, 8 h) gave the title troponoid (4) (0·60 g, 70%) as fine yellow needles, m.p. 152·5–156° (lit.[11] 155–157°) (Found: $M^{+\bullet}$, 343·1420. Calc. for $C_{19}H_{21}NO_5$: $M^{+\bullet}$, 343·1420). ^1H n.m.r. (400 MHz) δ 8·09, s, 1H, H8;† 7·51, d, *J* 12·0 Hz, 1H; 7·31, d, *J* 12·0 Hz, 1H; 6·55, s, 1H, H4; 3·91, s, 3H, OCH_3; 3·90, s, 3H, OCH_3; 3·82, m, 1H, H7; 3·67, s, 3H, OCH_3; 2·50–2·25, complex m, 3H; 1·75, m, 1H (OH and NH_2 not observed). ^{13}C n.m.r. (100 MHz) δ 172·1, 168·4, 153·6, 153·5, 150·4, 141·6, 141·1, 135·7, 135·5, 125·7, 123·8, 118·5, 106·9, 61·1, 61·0, 56·0, 53·5, 41·7, 30·5. ν_{max} (KBr) 3428, 2934, 1594, 1504, 1485, 1454, 1348, 1139, 1094 cm^{-1}. Mass spectrum *m/z* (70 eV) 343 (100%, M), 328 (32, $M-CH_3$), 312 (61, $M-CH_3O$), 298 (17), 207 (38). λ_{max} ($CHCl_3$) 245, 352 nm, $\log\epsilon$ 4·5, 4·3. $[\alpha]_D$ ($CHCl_3$) −109·8° (*c*, 8 mM).

N-*Chlorodeacetylcolchiceine (5)*

A mixture of compound (4) (2·58 g, 7·5 mmol), acetic acid (1·3 ml) and water (26 ml) was stirred at room temperature for 0·1 h until a homogeneous solution was obtained. A solution of *N*-chlorosuccinimide (1·1 g, 8·2 mmol) in acetic acid (11 ml) maintained at 60° was added. The reaction mixture was stirred at room temperature for 0·25 h. The resulting cream coloured solid was removed by vacuum filtration, washed with water (20 ml) and dried (90°/0·5 mm Hg, 6 h). Tropolone (5) was thus obtained as a brown solid (1·58 g, 56%),

† The colchicine (1) numbering scheme has been used for all compounds reported herein.

[22] Banwell, M. G., Lambert, J. N., and Richards, S. L., *Aust. J. Chem.*, 1991, **44**, 939.

m.p. 169–175° (lit.[10] 170–174°) [Found: (M − H), 376·0952. Calc. for $C_{19}H_{19}{}^{35}ClNO_5$: (M − H), 376·0952]. ^{1}H n.m.r. (400 MHz) δ 7·98, s, 1H, H8; 7·57, d, J 12·0 Hz, 1H; 7·34, d, J 12·0 Hz, 1H; 6·56, s, 1H, H4; 3·93, s, 3H, OCH_3; 3·92, s, 3H, OCH_3; 3·90, m, 1H, H7; 3·61, s, 3H, OCH_3; 2·60–1·80, complex m, 4H (OH and NH not observed). ν_{max} (KBr) 3446, 2935, 1595, 1544, 1488, 1458, 1404, 1347, 1268, 1228, 1138, 1094 cm^{-1}. Mass spectrum m/z (70 eV) 378 (1·5%), 376 (<1, M − H), 342 (100, M–Cl), 311 (59, M − CH_3O − Cl). λ_{max} ($CHCl_3$) 245, 345, 380 nm, log ϵ 4·6, 4·3, 4·0.

7-Oxodeacetamidocolchiceine (7)

The *N*-chloro compound (5) (1·44 g, 3·8 mmol) was dissolved in methanolic potassium hydroxide (8 ml of a 2 M solution) and the resulting solution was stirred at room temperature overnight. Water (10 ml) was then added and the solution adjusted to pH 6·5 by addition of acetic acid. Filtration of the ice-cold solution gave a brown solid which was washed with water (10 ml) and cold acetone (10 ml) to afford impure imine (6) (1·0 g). ^{1}H n.m.r. (400 MHz) δ 8·09, s, 1H, H8; 7·51, d, J 12·0 Hz, 1H; 7·31, d, J 12·0 Hz, 1H; 6·56, s, 1H, H4; 3·91(3), s, 3H, OCH_3; 3·91(0), s, 3H, OCH_3; 3·67, s, 3H, OCH_3; 2·52–2·18, complex m, 3H; 1·78, m, 1H (OH and NH not observed).

The crude imine (6) was dissolved in acetic acid (10 ml of a 50% aqueous solution) and heated at 50° for 0·75 h. Slow addition of water (10 ml) afforded a brown solid which was filtered off, washed with water (10 ml) and then recrystallized (acetic acid/water) to give the title troponoid (7) (0·31 g, 24%), m.p. 156–159° (lit.[12] 154°) (Found: $M^{+\bullet}$, 342·1103. Calc. for $C_{19}H_{18}O_6$: $M^{+\bullet}$, 342·1103). ^{1}H n.m.r. (400 MHz) δ 7·56, d, J 12·2 Hz, 1H; 7·39, d, J 12·2 Hz, 1H; 7·19, s, 1H, H8; 6·58, s, 1H, H4; 3·90, s, 3H, OCH_3; 3·89, s, 3H, OCH_3; 3·58, s, 3H, OCH_3; 3·19–2·85, complex m, 3H; 2·69, m, 1H (OH not observed). ^{13}C n.m.r. (100 MHz) δ 206·4, 175·1, 166·3, 154·0, 151·8, 147·0, 143·4, 141·7, 135·1, 134·1, 128·3, 124·7, 116·5, 106·9, 61·2, 61·1, 56·1, 48·8, 29·4. ν_{max} (KBr) 3436, 3184, 2938, 1694, 1595, 1560, 1460, 1397, 1352, 1336, 1274, 1233, 1139, 1095 cm^{-1}. Mass spectrum m/z (70 eV) 342 (100%, M), 314 (37, M − CO). λ_{max} ($CHCl_3$) 260, 350 nm, log ϵ 4·8, 4·2.

Methylation of compound (7) with diazomethane afforded a *c.* 1:1·2 mixture of compounds (2) and (3). These compounds were identical, in all respects, to the same compounds obtained by the more efficient methods detailed below.

Deacetylcolchicine (8) and Deacetylisocolchicine (9)

A magnetically stirred suspension of the troponoid (4) (0·20 g, 0·6 mmol) in dichloromethane (30 ml) was treated with ethereal diazomethane.[12] On completion of the reaction (t.l.c.) the resulting solution was concentrated to a glassy yellow solid. Subjection of this residue to radial chromatography (acetone/dichloromethane, 1:9) afforded two distinct chromophoric fractions.

Concentration of the more mobile fraction (R_F 0·2) afforded compound (9) (0·11 g, 51%) as a yellow oil with spectral characteristics matching those reported previously.[23] Various attempts to crystallize (9) were unsuccessful.

Concentration of the less mobile fraction (R_F 0·1) afforded compound (8) (0·09 g, 42%) as a yellow oil with spectral characteristics matching those reported previously.[23] Various attempts to crystallize (8) were unsuccessful.

7-Oxodeacetamidocolchicine (2) and 7-Oxodeacetamidoisocolchicine (3)

The crude mixture of *O*-methylated troponoids (8) and (9), obtained (see above) by treating (4) with diazomethane, was dissolved in a 3:1 mixture of dichloromethane/*N*,*N*-dimethylformamide (20 ml). 4-Formyl-1-methylpyridinium *p*-toluenesulfonate (0·53 g, 1·8 mmol, 1·2 equiv.) was added to the solution and the resulting mixture was heated at reflux for 2·5 h. The magnetically stirred solution was cooled in an ice-water bath and dbu was added dropwise to afford a deep-purple solution. Oxalic acid (25 ml of a 5% w/v aqueous solution) was added to the reaction mixture and vigorous stirring continued at room temperature for 1·5 h. The organic and aqueous fractions were separated and the aqueous phase was extracted with dichloromethane (3×20 ml). The combined organic phases were dried

[23] Hufford, C. D., Capraro, H.-G., and Brossi, A., *Helv. Chim. Acta*, 1980, **63**, 50.

($MgSO_4$) and then concentrated to an orange solid which was subjected to m.p.l.c. (silica, acetone/dichloromethane, 1:9) and two chromophoric bands were thereby obtained.

Concentration of the more mobile band [R_F 0·2(8)] afforded compound (3) (0·21 g, 39%) as light yellow solid. Recrystallization (ethyl acetate/petroleum spirit) of this material afforded a spectroscopically pure sample of (3) as yellow crystals, m.p. 193–196° (lit.[12] 190–192°) (Found: $M^{+\bullet}$, 356·1259. Calc. for $C_{20}H_{20}O_6$: $M^{+\bullet}$, 356·1260). The spectral data obtained for (3) matched those reported previously.[12,24] ^{1}H n.m.r. (400 MHz) δ 7·35, d, $J_{11,12}$ 12·8 Hz, 1H, H11; 7·22, d, $J_{12,11}$ 12·8 Hz, 1H, H12; 6·66, s, 1H, H8; 6·59, s, 1H, H4; 3·98, s, 3H, OCH_3; 3·91, s, 3H, OCH_3; 3·88, s, 3H, OCH_3; 3·63, s, 3H, OCH_3; 3·19, td, J 13·0, 6·0 Hz, 1H; 3·02, ddd, J 17·0, 6·0, 5·0 Hz, 1H; 2·94, ddd, J 17·0, 13·0, 4·0 Hz, 1H; 2·68, ddd, J 13·0, 5·0, 4·0 Hz, 1H. ^{13}C n.m.r. (100 MHz) δ 207·5, 179·4, 163·8, 153·8, 151·8, 141·6, 141·5, 141·4, 136·0, 135·0, 134·4, 124·3, 110·6, 106·7, 61·1, 61·0, 56·3, 56·0, 49·3, 29·4. ν_{max} (KBr) 1698, 1615, 1577, 1563, 1492, 1459, 1404, 1329, 1272, 1244, 1226, 1146, 1096 cm^{-1}. Mass spectrum m/z (70 eV) 356 (88%, M), 328 (100, M – CO).

Concentration of the less mobile band [R_F 0·2(6)] afforded compound (2) (0·18 g, 34%) as a light yellow solid. Recrystallization (ethyl acetate) of this material afforded a spectroscopically pure sample of (2) as yellow crystals, m.p. 231–234° (lit.[9] 232°) (Found: $M^{+\bullet}$, 356·1259. Calc. for $C_{20}H_{20}O_6$: $M^{+\bullet}$, 356·1260). The spectral data obtained for (2) matched those reported previously.[9,12,24] ^{1}H n.m.r. (400 MHz) δ 7·25, d, $J_{12,11}$ 11·1 Hz, 1H, H12; 7·14, s, 1H, H8; 6·86, d, $J_{11,12}$ 11·1 Hz, 1H, H11; 6·56, s, 1H, H4; 4·02, s, 3H, OCH_3; 3·89, s, 3H, OCH_3; 3·88, s, 3H, OCH_3; 3·57, s, 3H, OCH_3; 3·13, td, J 14·0, 5·0 Hz, 1H; 2·97, ddd, J 17·0, 5·0, 3·0 Hz, 1H; 2·84, ddd, J 17·0, 14·0, 5·0 Hz, 1H; 2·70, ddd, J 14·0, 5·0, 3·0 Hz, 1H. ^{13}C n.m.r. (100 MHz) δ 205·6, 179·4, 165·0, 153·8, 151·9, 150·1, 141·7, 136·4, 135·4, 132·9, 132·0, 124·6, 112·3, 107·0, 61·2, 61·1, 56·5, 56·0, 47·4, 29·3. ν_{max} (KBr) 1707, 1618, 1581, 1489, 1468, 1433, 1407, 1396, 1350, 1273, 1252, 1224, 1137, 1094 cm^{-1}. Mass spectrum m/z (70 eV) 356 (75%, M), 328 (100, M – CO).

Single-Crystal X-Ray Diffraction Analysis of Compounds (2) and (3)

Accurate unit cell dimensions were determined at 291(1) K by least squares refinement for 25 automatically centred reflections in the range $30° < 2\theta < 62°$ measured with Cu Kα (λ 1·5418 Å) radiation. Intensity data were measured on a Rigaku-AFC diffractometer with Cu Kα radiation (graphite-crystal monochromator) and were recorded by an ω–2θ scan, 2θ scan rate 2° min^{-1}, scan range ($\Delta\omega$) 1·2°+0·5°tanθ, and 10 s stationary background counts. Three standard reflections monitored every 50 reflections showed no significant variation. Data were recorded to a $2\theta_{max}$ 120° for (2), 130° (3) and terms for which $I \geq 2\sigma I$ were used for the structure refinements. Corrections were made for Lorentz and polarization factors; analytical absorption corrections were made with SHELX76[25] [transmission factors from 0·923 to 0·784 (2), and from 0·846 to 0·706 (3)].

Crystal Data

Compound (2).—$C_{20}H_{20}O_6$, M 356·4, triclinic, space group $P\bar{1}$, a 7·416(2), b 10·498(2), c 12·772(3) Å, α 66·35(2), β 71·60(1), γ 82·71(2)°, V 864·3(4) Å^3, D_m(flotation) 1·36(1), $D_c(Z = 4)$ 1·369 g cm^{-3}, F(000) 376, μ(Cu Kα) 7·99 cm^{-1}. A pale yellow tabular crystal from ethyl acetate with dimensions c. 0·10 by 0·38 by 0·26 mm used for data collection (h from −8 to 8, k from −11 to 11, l from 0 to 14) yielded 1741 unique terms. Final R 0·042 ($\sum\Delta F/\sum|F_o|$ where $\Delta F = ||F_o| - |F_c||$), wR 0·052 $[w(\Delta F)^2/\sum w|F_o|^2]^{1/2}$, with $w = [\sigma^2|F_o|+0·00085|F_o|^2]^{-1}$ and S 1·29$[\sum w(\Delta F)^2/(N_o - N_v)]^{1/2}$ for 1231 (N_o) data and 237 variables (N_v). At convergence $(\Delta/\sigma)_{max}$ = 0·002, and $(\Delta\rho)_{max}$ and $(\Delta\rho)_{min}$ were +0·17 and −0·21 e Å^{-3}, respectively.

Compound (3).—$C_{20}H_{20}O_6$, M 356·4, monoclinic, space group Cc, a 16·796(4), b 10·174(2), c 10·957(2) Å, β 111·12(2)°, V 1747(7) Å^3, D_m(flotation) 1·36(1), $D_c(Z = 4)$ 1·355 g cm^{-3}, F(000) 752, μ(Cu Kα) 7·91 cm^{-1}. A pale yellow tabular crystal with dimensions c. 0·27 by 0·38 by 0·41 mm from ethyl acetate/petroleum spirit used for data collection (h

[24] Delaroff, V., and Rathle, P., *Bull. Chim. Soc. Fr.*, 1965, 1621.
[25] Sheldrick, G. M., SHELX76, Program for Crystal Structure Determination, University of Cambridge, Cambridge, U.K., 1976.

from −20 to 20, k from 0 to 12, l from 0 to 12) yielded 1568 unique terms. Final R 0·035, wR 0·044 ($w = [\sigma^2|F_o|+0·0006|F_o|^2]^{-1}$), S 1·57 (299 variables and 1416 data). At convergence $(\Delta/\sigma)_{max}$ = 0·004, and $(\Delta\rho)_{max}$ and $(\Delta\rho)_{min}$ were +0·18 and −0·19 e Å^{-3}, respectively.

Structure Determination

The structures were solved by direct methods with SHELXS[26] for (2) and with XTAL3.0[27] for (3). Full-matrix least-squares refinements were carried out with SHELX76[25] on a VAX8800 computer. Because of the paucity of data for (2), the hydrogen atoms were included at idealized positions; the methyl and non-methyl hydrogen atoms were given common isotropic temperature factors which refined to values of 9·8(5) and 4·5(4) Å^2, respectively. For (3), the C(1M) methyl hydrogen atoms were included at idealized positions and given a common isotropic temperature factor [18(2) Å^2]; the parameters (x, y, z, U_{iso}) of the other hydrogen atoms were refined, apart from H(2Mβ) which was held fixed in the found position. Anisotropic temperature factors were given to the non-hydrogen atoms in (2) and (3) and the function minimized was $\sum w(|\Delta F|)^2$. An isotropic extinction correction of the form $F_c = F[1-2·58(3)\times10^{-6}|F|^2/\sin\theta]$ was applied to the calculated structure amplitudes of (2), whereas four intense low order terms ($\bar{1}$ 1 2, $\bar{2}$ 2 2, $\bar{1}$ 1 3, 0 0 2) seriously affected by extinction were omitted from the final refinement of (3). Neutral scattering factors[28] were used, those for carbon and oxygen being corrected for anomalous dispersion.[29] The results are presented in Tables 1–4 and Fig. 1. The last was prepared from the output of ORTEPII[30] and contains the atomic numbering. Material deposited: anisotropic thermal parameters, hydrogen atom parameters, bond lengths and angles, and observed and calculated structure amplitudes.†

Biological Studies

Materials.—Tubulin was purified from bovine brain as described previously.[31] Colchicine (1) was obtained from Sigma Chemical Company. Compounds (13) and (14) were generously provided by Professor A. Brossi of the National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland. Monosodium glutamate, from Sigma, was repurified by acid precipitation and reneutralization (to pH 6·6, in a 2 M stock solution) with ultrapure NaOH obtained from Alfa.[32]

Tubulin polymerization assay.—Each reaction mixture was contained in a 0·24 ml volume 1·0 mg/ml (10 μM) tubulin, 1·0 M monosodium glutamate, 1·0 mM $MgCl_2$, 4% (v/v) dimethyl sulfoxide, and varying concentrations of drugs (all concentrations, however, refer to the final reaction volume of 0·25 ml). Reaction mixtures were preincubated to 37° for 15 min and chilled on ice, and 10 μl of 10 mM guanosine 5′-(tetrahydrogen triphosphate) (required for polymerization) was added to each mixture. Reaction mixtures were transferred to cuvettes in Gilford spectrophotometers held at 0° by electronic temperature controllers. Baseline absorbances at 350 nm were established, and the reaction was initiated by a temperature jump to 37° (the temperature rose at a rate of about 0·5°/s). The reactions were followed for 20 min, and IC_{50} values, defined as drug concentration required to inhibit the extent of polymerization by 50% after a 20 min incubation, were determined graphically. At least three

† Copies are available on application to the Australian Journal of Chemistry, P.O. Box 89, East Melbourne, Vic. 3002.

[26] Sheldrick, G. M., SHELXS, in 'Crystallographic Computing 3' (Eds G. M. Sheldrick, C. Krüger and R. Goddard) (Oxford University Press: London 1985).
[27] Hall, S. R., and Stewart, J. M., (Eds) "The XTAL User's Manual—Version 3.0", Universities of Western Australia and Maryland, 1990.
[28] Ibers, J. A., and Hamilton, W. C., (Eds) 'International Tables for X-Ray Crystallography' Vol. 4, p. 99 (Kynoch Press: Birmingham 1974). Present distributor Kluwer Academic Publishers, Dordrecht.
[29] Cromer, D. T., and Liberman, D. J., *J. Chem. Phys.*, 1970, **53**, 1981.
[30] Johnson, C. K., ORTEPII, Report ORNL-5138, Oak Ridge National Laboratory, Tennessee, U.S.A., 1976.
[31] Hamel, E., and Lin, C. M., *Biochemistry*, 1984, **23**, 4173.
[32] Huang, A. B., Lin, C. M., and Hamel, E., *Biochim. Biophys. Acta*, 1985, **832**, 22.

independent experiments were performed with each drug, except that inactive compounds (defined as IC_{50} >40μM) were generally evaluated only twice. Four spectrophotometers (16 samples) were used in each experiment. Each experiment had two control reaction mixtures, with the turbidity readings generally within 5% of each other. Repurified glutamate was used in the studies presented here, as opposed to commercial glutamate used in previous work.[5,21] This modification has resulted in significantly lower IC_{50} values with all agents thus far examined [compare values obtained here with colchicine, (13) and (14) with those earlier[21]]. The presumptive contaminant in commercial glutamate causing reduced inhibitory effects is unknown.

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