CONTRACTION OF THE TROPOLONIC RING OF COLCHICINE BY HYDROGEN PEROXIDE OXIDATION

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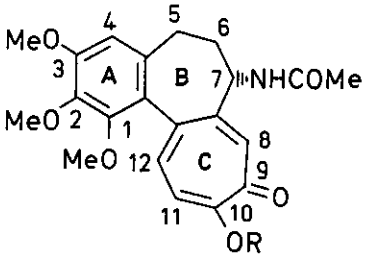
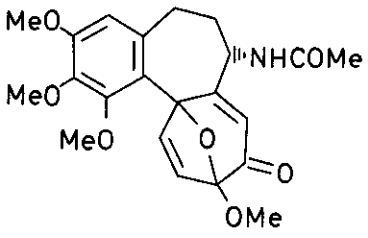
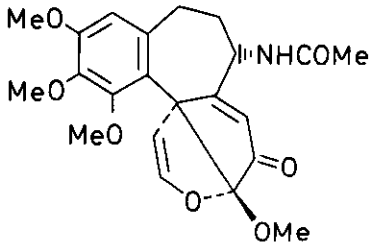
This paper is dedicated to Dr. Arnold Brossi on the occasion of his 60th birthday

Abstract - By hydrogen peroxide oxidation of colchicine the tropolonic ring underwent contraction to a benzene ring. Two compounds were obtained and identified (EIMS, ^{1}H NMR) as N-acetylcolchinol methyl ether and 10-carbomethoxy-N-acetyl-colchinol. Antimitotic activity is also reported.

The tropolonic ring of colchicine (1) has been shown to suffer oxidative attack with chromic acid,[1,2] or with aqueous sodium peroxide [3] without ring contraction, the products obtained being 2 and 3 respectively. Oxidative attack of colchiceine (4) by hydrogen peroxide in alkaline medium has been reported to cause contraction of the tropolonic ring, the resulting compound being N-acetylcolchinol (5).[4] Colchicine easily dissolved in 30% hydrogen peroxide and the homogeneous solution, when heated under magnetic stirring on an oil bath at 75-80 °C for 32 h, in a dim light, is transformed in a thick yellow oil which separated from the aqueous phase. Stirring at this point became difficult; at the same time, TLC revealed only traces of colchicine. The glassy oil was extracted with chloroform and passed through a short column of silica gel, with chloroform containing 2.5% methanol and 0.5% ammonia as the eluent, to remove impurities from the main fraction, which solidified after this passage. It gave only one spot on TLC on SiO_2 F254, $CHCl_3$-MeOH-NH_4OH 90:9:1, R_f 0.63 (corresponding R_f values were: 1, 0.48; 2, 0.55; 3, 0.58). When the same product was developed with $CHCl_3$-Me_2CO-$NH(Et)_2$ 5:4:1, it gave two



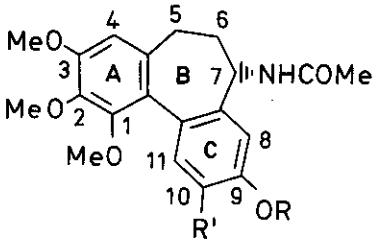




1. R = Me, colchicine
4. R = H , colchiceine

2. 10,12a-epoxycolchicine (oxycolchicine)

3. 10,11-oxy-10,12a-cyclo 10,11-seco-colchicine



5. R = R' = H, N-acetylcolchinol
6. R = Me, R' = H, N-acetylcolchinol methyl ether
7. R = H, R' = COOMe, 10-carbomethoxy-N-acetylcolchinol
8. R = Me, R' = COOMe

distinct spots: (6), R_f 0.65; (7), R_f 0.57; the latter showing a blue fluorescence under UV light (corresponding R_f values were 1, 0.59; 2, 0.62; 3, 0.64). The two compounds were separated by fractional crystallization from ethyl acetate, that gave only (6) in a pure form, while (7) was further purified and liberated from (6) by column chromatography on silica gel-60 (0.04-0.06 mm), using chloroform containing 0.25% diethylamine as the eluent. Both compounds sublimed at 150-155°C/0.005 mm in a ball tube oven, and crystallized from ethyl acetate as white elongated needles. Approximately the amount of (6) was more than twice that of (7). The first eluted compound (6), under electron impact mass fragmentation, showed the molecular ion at m/z 371 (base peak); this ion represents the first fragment in the mass spectrum of colchicine,[5] originating from the expulsion of CO from the molecular ion 399. Further fragmentation either of colchicine or of (6) proceeded similarly. On the basis of these spectral data (elemental analysis was in agreement with the formula $C_{21}H_{25}NO_5$), compound (6) was assigned the structure of N-acetylcolchinol methyl ether, a well-known compound that was not yet obtained directly from colchicine. Its mp 202-204 °C and its MS corresponded to those reported for (6) prepared by a different route;[4,6] $[\alpha]_D^{20}$ - 64.9 ± 0.7° (c, 1.03%, $CHCl_3$).[7]

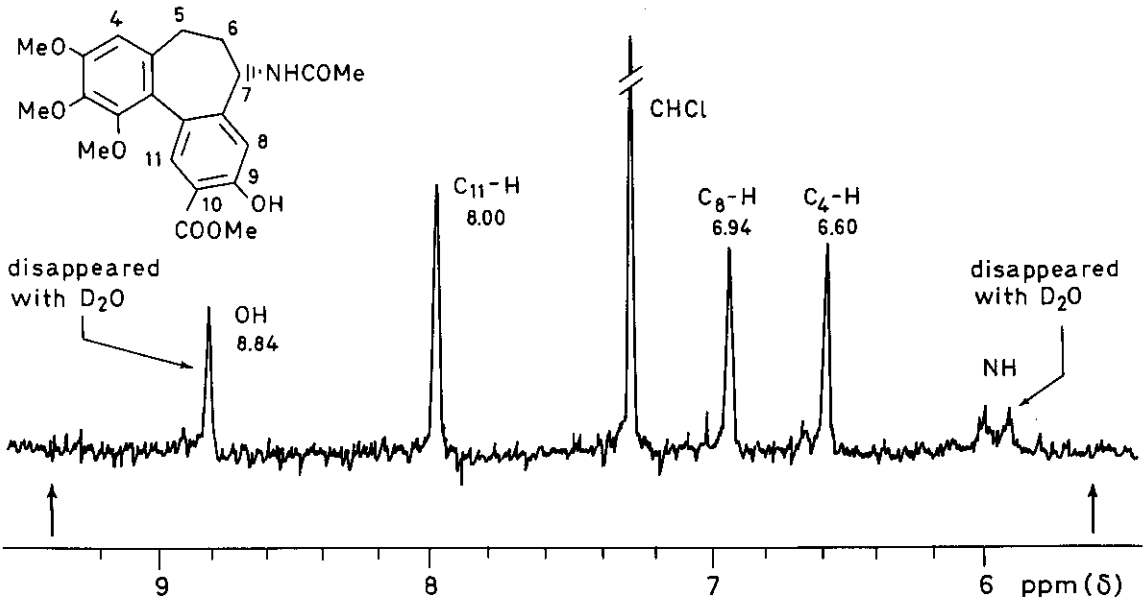
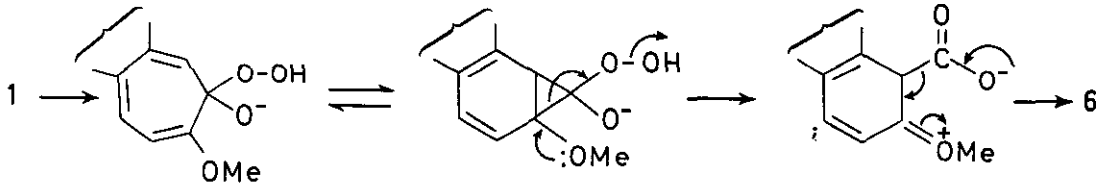


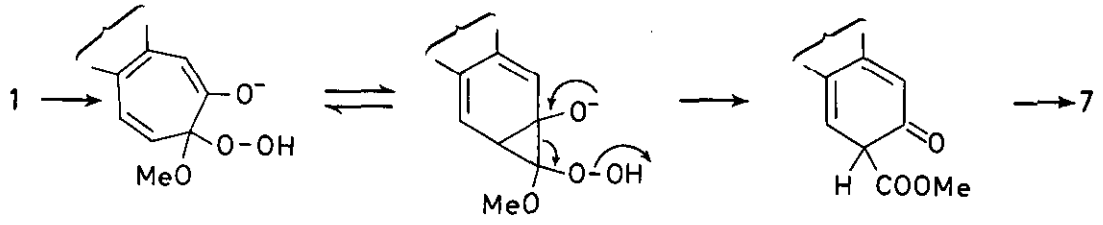
Fig. 1 - Aromatic region of ^{1}H NMR spectrum (Varian XL-100) of 10-carbomethoxy-N--acetylcolchinol in $CDCl_3$.

The second eluted compound (7) showed the molecular ion at m/z 415, corresponding to the presence of an additional oxygen in the molecule of colchicine. It showed the same molecular weight and elemental analysis ($C_{22}H_{25}NO_7$) as those of 2 and 3, but different TLC behaviour, mass and ^{1}H NMR spectra. Its structure as 10-carbomethoxy-N-acetylcolchinol was based mainly on the interpretation of its ^{1}H NMR spectrum. In the low frequency region (Fig. 1) the singlet at δ 6.60, present in all colchicine-like structures examined,[8,9] was assigned to the aromatic C-4-H of ring A; the remaining signals of the aromatic region appeared also as singlets, and hence represented isolated protons in agreement with ring C structure indicated in 7. Furthermore, the signal at lowest field was assigned to the phenolic proton, since it disappeared by deuterium exchange. In the EI mass spectrum the molecular ion, M^+ 415, represented the base peak. This is an additional evidence of the contraction of ring C: in fact, in colchicine-like compounds with a tropolonic ring,[10] the molecular ion never represented the base peak; stabilization of the molecule being attained only after expulsion of CO. Other fragments are at m/z (%): 383 (26), 356 (58), 324 (82), 313 (56), 43 (74). IR (KBr): ν C=O (amide) 1645 cm^{-1}, ν C=O

Scheme 1



Scheme 2



(ester, hydrogen bonded with the vicinal OH) 1680 cm^{-1}; $[\alpha]_D^{20}$ - 5.0 ° (c, 1.04%, $CHCl_3$); mp 211-212 °C (from ethyl acetate). O-Methylation of 7 with dimethyl sulfate and potassium carbonate in acetone, at reflux temperature, gave the C-9 methyl ether 8, as an amorphous powder (M^+ 429, base peak).

The formation of 6 can be expected to occur by nucleophilic attack of the hydroperoxide anion on the carbonyl carbon, valence-bond tautomerization to a norcaradiene-type derivative [11] followed by carbon dioxide expulsion (Scheme 1).

Formation of 7 can be understood by consideration of the mechanism outlined in Scheme 2: addition of the hydroperoxide anion to the electrophilic C-10, tautomerization to a norcaradiene-type intermediate followed by rearrangement.

Compounds 6 and 7 were tested for their antimitotic activity in the tubulin binding assay [12] together with reference compounds (Table). This *in vitro* assay represents a valuable screening for further investigation of antitumor activity.[10] Compound 7, with an affinity of 46% should result almost inactive in the antileukemic P 338 screen, while 6 is a worthy candidate for this *in vivo* assay.[12]

Table. Inhibition of colchicine binding to rat brain tubulin by colchicine analogs

Compounds	% Inhibition of ^{3}H-colchicine-tubulin binding [a]
Colchicine (1)	89
N-acetylcolchinol methyl ether (6)	100
10-Carbomethoxy-N-acetylcolchinol (7)	46
Allocolchicine [b]	81
N-acetylcolchinol (5)	52 [c]

[a] Percentage by which binding is reduced by the presence of the inhibitor at 2.5×10^{-5} M, with ^{3}H-colchicine at 2.5×10^{-6} M.

[b] Allocolchicine or colchicic acid methyl ester possesses a benzene ring C with a carbomethoxy group at C-9.

[c] From M.H. Zweig and C.F. Chignell, Biochem. Pharmacol., 1973, 22, 2141.

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