REACTION OF COLCHICEINAMIDE WITH PHOSGENE AND WITH THIOPHOSGENE: STRUCTURES AND ANTITUBULIN ACTIVITY OF TETRACYCLIC OXAZOLONES, OXAZOLETHIONES AND THIAZOLONES OF THE COLCHICINE SERIES

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Abstract - Colchiceinamide (5) on reaction with phosgene and thiophosgene in the presence of triethylamine afforded tetracyclic compounds (7) and (8) respectively. Treatment of oxazolethione (8) with sodium hydroxide afforded thiazolone (9). All three tetracyclic compounds had very high negative specific rotations, but none of them inhibited tubulin polymerization in vitro.

Elucidation of the peptide substructure which comprises the colchicine binding site on tubulin is pivotal for a better understanding on how colchicine and other spindle toxins work, and how they disrupt assembly of the microtubular network. Attempts to solve this problem suggested the design of colchicinoids marked with a highly reactive group. It is thought that such a compound would, on reaction with a prosthetic group on the protein, forma covalent bond, allowing determination of its location by amino acid sequencing.

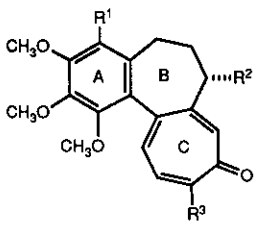
The isothiocyanato group " -NCS", introduced at a non-critical carbon atom was successfully used by Rice to map and to separate opioid receptor subpopulations.[1] Therefore we thought it worth while to introduce an isothiocyanato group in colchicine (1) and isothiocyanates (2), (3) and (4) became the immediate target molecules. The chemistry and biological properties of the compounds obtained in our attempts to

make 3 are reported here; synthesis and properties of markers (2) and (4) will be reported elsewhere.

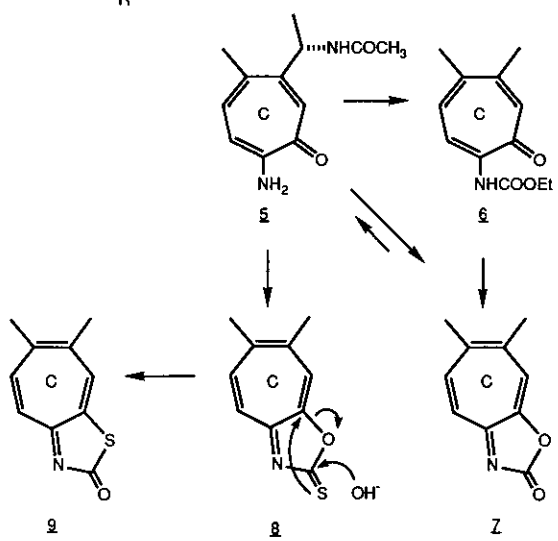
Reaction of colchiceinamide (5) with phosgene and thiophosgene in the presence of triethylamine afforded oxazolone (7) and thio-isostere (8) respectively. Both structures are supported by spectral data, particularly the ir and uv spectra respectively which show characteristic signals assigned to CO and CS groups, and a bathochromic shift of the uv maximum by 15 nm when the N=CO group is converted into a N=CS group[2].

Oxazolone (7) is also obtained as a side product in the preparation of carbamate (6). It is assumed that carbamate (6) undergoes cyclization, a reaction also observed with carbamate intermediates in Eschenmoser's total synthesis of colchicine[3]. Compounds (7) and (8) show unusually high specific optical rotations (427° and 1204° respectively) when measured in chloroform/MeOH (1:1) solution, suggesting that the aromatic ring A and rings C/D representing the biaryl system are non-coplanar[4].

Treatment of 7 with 1N sodium hydroxide afforded amide (5) but similar treatment



1 R^1=H, R^2=NHCOCH$_3$, R^3=OCH$_3$
2 R^1=H, R^2=NCS, R^3=SCH$_3$
3 R_1=H, R^2=NHCOCH$_3$, R^3=NCS
4 R^1=NCS, R^2=NHCOCH$_3$, R^3=SCH$_3$



of 8 afforded, unexpectedly, thiazolone (9) . Structure (9) is proposed on the basis of spectral data, showing the presence of the imide CO group in the ir spectrum at 1670 cm^{-1} and the disappearance of the CS group in 8 at 1300 cm^{-1}. It can be speculated that thiazolone (9) derives from oxazolethione (8) on hydrolysis.

BIOLOGICAL DATA:Compounds (7), (8) and (9) did not inhibit the polymerization of purifiedtubulin at IC_{50} values of 100 μM (colchicine, IC_{50}=2.4 μM)[5].

EXPERIMENTAL

The optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at temperature range 22-25°C. The uv spectra (λ_{max}, EtOH) were measured on a Hewlett-Packard 8450 A uv/vis spectrophotometer. The ir spectra (υ_{max} , $CHCl_3$) were recorded on a Beckman IR 4230 instrument. The ^{1}H-nmr spectra were measured on a Varian XL-300 spectrometer. Electron impact mass spectra were determined on a Finnigan 1015D spectrometer with a model 6000 data collection system. Thin layer chromatography plates were purchased from Analtech Inc., Newark, DE and silical gel 60 (230-400 mesh) from Fluka was used for column chromatography.

Oxazolone 7: To a solution of colchiceinamide[6] (5, 100 mg, 0.26 mmol) in methylene chloride (4 ml) and triethylamine (0.5 ml) was added phosgene (0.02 ml of 20% in toluene) under ice-cooling. The reaction mixture was stirred under nitrogen at room temperature for 2 h then diluted with methylene chloride, washed with 5% HCl and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under high vacuum. The crude extract was flash chromatographed over silica gel using chloroform/ methanol (98:2) as eluant to give pure oxazolone (7) as yellow powder (50 mg , 47%): $[\alpha]_D$ -427° (c=0.16, $CHCl_3$); ms (m/z) 410 (M^+), 382 (100%), 367, 351, 339, 323, 308, 292, 278, 265; ir 1780, 1750 (C=O, imide), 1650 cm^{-1} (C=O, amide); uv 257 and 391 nm ^{1}H-nmr: ($CDCl_3$, δ) 2.22 (3H, s, $COCH_3$), 3.78 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.09 (3H, s, OCH_3), 4.85 (1H, s, H-7), 6.74 (1H, s, H-4), 7.90 (1H, d, J=6.47, NH), 8.05 (1H, d, J=11.7 Hz, H-11), 8.25 (1H, d, J=11.7 Hz, H-12), 8.40 (1H, s, H-8).

Oxazolethione 8: To a solution of colchiceinamide (5) (100 mg, 0.26 mmol) in methylene chloride (4 ml) and triethylamine (0.5 ml) was added thiophosgene (0.2 ml) under ice-cooling. The reaction mixture was stirred under nitrogen at room

temperature for 2 h, diluted with methylene chloride, washed with 5% HCl, saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to give 106 mg of crude product. The product was flash chromatographed on silica gel using chloroform/methanol (9:1) as eluant to give an orange amorphous material (8, 45mg, 41%): $[\alpha]_D$ -1204° (c=0.14, 9:1 $CHCl_3$/MeOH); ms 426 (M^+, 100%), 398, 383, 367, 352, 339, 324, 308, 296, 281, 266; ir 1670 (C=O, amide), 1300 cm^{-1} (C=S); uv 256 and 460 nm; ^{1}H-nmr ($CDCl_3$, δ) 2.00 ($COCH_3$), 3.58 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.65 (1H, m, H-7), 6.54 (1H, s, H-4), 7.30 (1H, d, J=5.74, NH), 8.00 (1H, d, J=11.6 Hz, H-11), 8.12 (1H, d, J=11.6 Hz, H-12), 8.18 (1H, s, H-8).

Hydrolysis of oxazolone (7) to colchiceinamide (5): Oxazolone (8, 20 mg) was dissolved in ethanol (2 ml) and then 2 drops of 10% NaOH was added. The solution was reacidified with 5% HCl and extracted with chloroform to give a single product (15 mg) which was found to be identical with colchiceinamide (tlc comparison, uv, mass spectra and melting point)[6].

Hydrolysis of oxazolethione (8) to thiazolone (9): To a solution of oxazolethione (8, 50 mg) in EtOH (5 ml) was added 10% NaOH (1 ml) at room temperature and stirred for 10 minutes. After acidification with 5% HCl and extraction with chloroform, an orange solid was obtained which was chromatographed on preparative silica gel plates using $CHCl_3$/MeOH/NH_4OH (90:9:1) as eluant to give thiazolone (9) as dark yellow powder (24 mg, 48%): $[\alpha]_D$ -671° (c=0.14, $CHCl_3$); eims (m/z) 426 (M^+, 100%), 398, 383, 367, 353, 341, 323, 308, 292; ir 1670 (C=O, imide), 1640 cm^{-1} (C=O, amide); uv 247, 408 nm; ^{1}H-nmr ($CDCl_3$, δ), 2.02 (3H, s, $COCH_3$), 3.59 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.63 (1H, m, H-7), 6.49 (1H, bs, NH), 6.52 (1H, s, H-4), 7.87 (2H, s, Ar-H), 8.18 (1H, s, Ar-H).

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