J. Pharm. Pharmacol. 1998, 50 (Supplement): 244

Gossypol and its derivatives as novel agents for the treatment of melanoma

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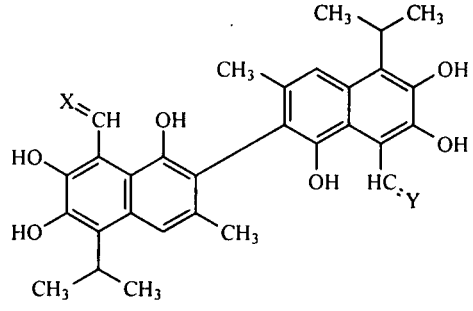
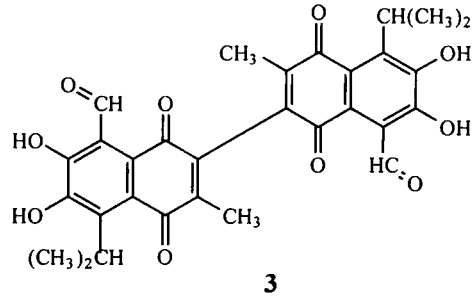
Gossypol (1,1',6,6',7,7' - hexahydroxy- 5,5' - diisopropyl- 3,3 '-dimethyl- 2,2' -binaphthalene- 8,8' -dicarbaldehyde) **1**, a natural product isolated from the pigment glands of the cotton plant (*Gossypium*), has been extensively studied for use as an oral contraceptive in Man. Although gossypol was subsequently withdrawn because of low frequency side-effects, its favourable toxicity profile, together with recent demonstrations of anti-tumour activity in animals and humans, Jaroszewski et al (1990), prompted us to investigate structure-activity relationships in cultured tumour cells. As a result of hindered rotation about the 2,2'-binaphthyl bond, gossypol exhibits atropisomerism and d-Gossypol exists in high enantiomeric excess in most plants from which gossypol is isolated but it is the l-isomer which generally has the greater biological activity.



1 X = Y= O

2 X = O, Y = $NCH(CH_2Ph)CO_2Et$

We have isolated the d- and l-atropisomers by Schiff's base formation using a chiral amine, isolation, and regeneration of the individual isomers by acid hydrolysis, Groundwater et al, 1995. Half-Schiff's bases **2** (one free aldehyde group), and a number of derivatives including the major metabolite in mammals, gossypolone **3**, were also prepared. The crystal structure of gossypolone shows that the binaphthyl dihedral angle is 79.2° and, using this structure as a starting point, the structure was minimised and the barriers to rotation about the 2,2'-bond were calculated. The energy barrier for the rotation which gives the conformation in which the two methyl and carbonyl groups approach one another (1873 kcal/mol) is significantly higher than that in which there are two methyl group-carbonyl group interactions (1100 kcal/mol).



3

Cell lines studied included melanomas (SK mel 19, SK mel 28, V39), lung (H69), mammary (Walker), cervix (SiHa) and leukaemia (K562, Molt-4). Cytotoxicity was determined using viability assays (flow cytometry or MTT) and the clonogenic assay. In the melanoma cell lines the % viable cell numbers remaining in serum-containing media after 4 days for each isomer of gossypol and the racemate are given in Table 1. The IC_{50} for l-gossypol in the cell lines studied ranged from 5-19μM. Cytotoxicity was associated with cell shrinkage, altered cell adhesion, and membrane blebbing. The l-isomer was more toxic than cisplatin, melphalan, and dacarbazine. Blocking or removing both of the aldehyde groups in gossypol resulted in no cytotoxic effect; in contrast, using a half-Schiff's base the cytotoxicity was similar to that of l-gossypol. d/l-Gossypolone was as cytotoxic as d/l-gossypol in some cell lines. Further studies on the mode of action are currently underway.

Table 1. % Viable cell numbers in melanoma cell lines treated with gossypol isomers (10μM).

Isomer	% viable (mean ± sem)
l	17 ± 4
d/l	49 ± 1
d	99 ± 0.8

Jaroszewski JW, Kaplan O, Cohen JS (1990) Cancer Res. 6936-6943.

Fish RG, Groundwater PW, Morgan JJG (1995) Tetrahedron Asymm. 873-876.