TRIPDIOLIDE FROM TISSUE CULTURE OF TRIPTERYGIUM WILFORDII.

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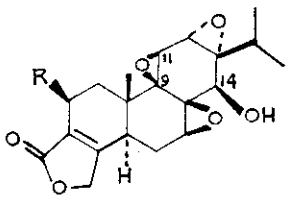
Abstract - Shake flask cultures of plant tissue cells from Tripterygium wilfordii have yielded the cytotoxic compounds tripdiolide and celastrol.

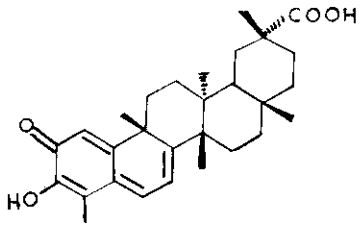
Tripdiolide (1) and triptolide (2) show significant activity in vivo against L-1210 and P-388 leukemia's in the mouse and in vitro against cells derived from human carcinoma of the nasopharynx (KB)[1]. Due to the difficulty associated with the collection of the plant in which they occur, (Tripterygium wilfordii) as well as their low concentration (0.001%), alternative sources are being sought. For example, synthetic studies directed toward the total synthesis of triptolide have commenced[2,3]. We now report the first production of tripdiolide (1) by tissue culture of the Tripterygium wilfordii plant.

Plant tissue culture cells were grown in modified B-5 and PRL-4 suspension media[4] in shake flasks for a period of 7 weeks. In a typical experiment, 5 l of medium yielded after extraction of the medium and cells with EtOAc, 4.8 g of crude product. Separation of this mixture on silica columns gave a yield of 0.003% of tripdiolide based on the dry cell weight. Identity was confirmed by mass spectrometry, 270 MHZ NMR and TLC comparison with an authentic sample. There was also TLC evidence for the presence of triptolide, and larger batches are being grown in order to get spectral confirmation. A programme involving fermenter scale production as well as maximisa-

tion of tripdiolide production by way of cell selection and media optimisation are now underway.



1. R = OH
2. R = H



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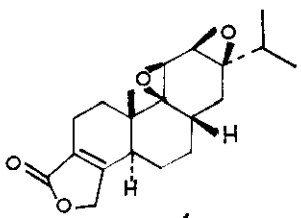

4.

Several other natural products have also been isolated. They include a number of triterpenes ($C_{30}H_{48}O_3$, $C_{30}H_{48}O_4$, $C_{30}H_{48}O_5$), β-sitosterol and two related compounds ($C_{29}H_{48}O$ and $C_{29}H_{46}O$). The cytotoxic triterpene celastrol (3) (KB evaluation) is also a major component of these tissue cells.

In 1974, Kupchan and co-workers studied the structure/activity relationship of some triptolides and concluded that the hydrogen bonded 9,11-epoxy-14β-hydroxy system was essential for cytotoxicity against KB cells in culture[5]. The fact that stemolide (4) from Stemodia maritima[6] is inactive in anti-tumor tests[7], supports this observation.

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