

CONVENIENT PREPARATION OF IRIDODIAL BY REDUCTION OF NEPETALACTONE.
CORROBORATION FOR THE ROLE OF IRIDODIAL AS AN INDOLE ALKALOID PRECURSOR IN A
CATHARANTHUS ROSEUS CELL SUSPENSION

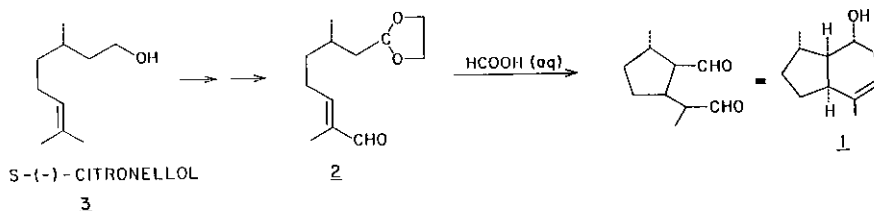
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Abstract - Iridodial of high isomeric purity was effectively prepared by reduction of nepetalactone with sodium borohydride in *tert*-butanol. Nepetalactone, in turn was readily obtained by steam distillation of the aerial parts of Nepeta cataria. Feeding of deuterium-labelled iridodial prepared as above and deuterium-labelled material prepared by acid-promoted cyclization of 10-oxocitronellal, to a Catharanthus roseus cell suspension culture, afforded the alkaloid, ajmalicine, which was specifically labelled with deuterium to the extent of 76% and 36%, respectively.

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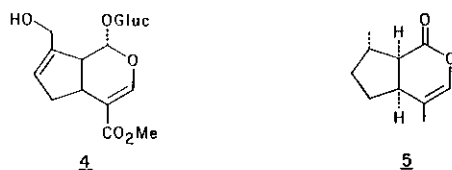
Iridodial (1) has been implicated as a common precursor to the group of cyclopentanoid terpenes known as iridoids^{1,2} as well as to monoterpene indole alkaloids³⁻⁷. In turn, 1 arises from the acyclic monoterpene(s) 10-oxogeraniol and/or 10-oxonerol. Interestingly, a number of 9,10-oxygenated derivatives of geraniol (but not nerol) had also been implicated as indole alkaloid precursors⁸.



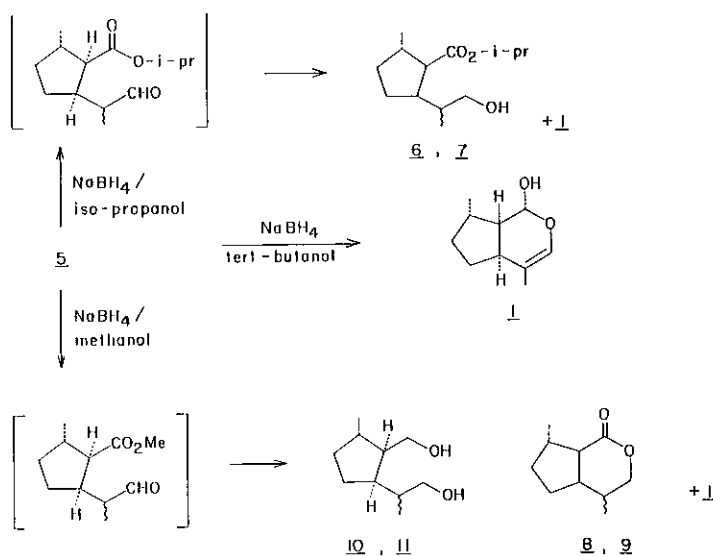
Scheme 1. Outline of Bowman and Leetes' preparation of iridodial.

As we had earlier prepared a number of 9,10-oxygenated geraniol and geraniol derivatives⁹, we now

sought a convenient preparation of iridodial in order to further pursue our interest in the early stages of indole alkaloid biosynthesis. Previously, Bowman and Leete¹⁰ had prepared labelled iridodial by cyclization, under acidic conditions, of the monoacetal of 10-oxocitronellal (2) (Scheme 1). By using S-(-)-citronellool (3) as the starting material, the stereochemistry of the C-3 methyl group was fixed, however this approach afforded little control over the stereochemistry at the carbons adjacent to the two formyl functions, due to the equilibrating conditions employed, and in our hands repetition of this reaction led to a complex mixture of "iridodials". This result may well have been a contributing factor to the lack of incorporation of this iridodial into Catharanthus indole alkaloids as originally observed by Bowman and Leete¹⁰. Conversely, Uesato et al.¹¹ prepared iridodial from the natural material geniposide (4) via a multistep sequence of reactions. This preparation was attractive from the points of view of stereochemical control and versatility; however, it suffered the drawback of being a non-trivial laborious sequence to



perform. Accordingly, we sought to develop a convenient and simple preparation of iridodial. Our approach was based on the reduction of the natural product nepetalactone (5), which could be readily obtained by steam distillation of the aerial parts of the catnip plant, Nepeta cataria. Although a number of isomeric nepetalactones have been isolated from Nepeta species,¹²⁻¹⁵ the steam-volatile oil obtained by us from field grown plants of N. cataria consisted of >90% 5 as determined by gas chromatography, proton magnetic resonance spectrometry, and carbon-13 magnetic resonance spectrometry. Reduction of 5 with sodium borohydride in tert-butanol afforded a single isomer of iridodial in the bicycle hemiacetal form with the stereochemistry known at all centres except for the newly generated lactol which was tentatively assigned the α -OH configuration as indicated in structure 1, Scheme 2. The course of the reduction was very sensitive to the solvent employed. With tert-butanol, the only significant product obtained after 20 h. was 1, which could be isolated in 49% yield. If instead of tert-butanol, isopropanol or methanol were used, only traces of 1 were observed (Scheme 2), with the major products now being the hydroxy isopropyl esters 6 and 7 in the first case, and the saturated lactones 8 and 9 and diols 10 and 11, in the second case. Clearly, when an alcohol less hindered than tert-butanol was used as solvent the rate of ring opening of the lactone of nepetalactone by the alcoholic solvent was more significant than its reduction, thus accounting for the formation of the products observed.



Scheme 2. Outline of products obtained from the sodium borohydride reduction of nepetalactone in various solvents.

Having developed this preparation of iridodial, the utility of this material as an indole alkaloid precursor was established by preparing the C-1 deuterium labelled analog using sodium borodeuteride as the reducing agent and using this material as a substrate with a *Catharanthus roseus* cell suspension to check for incorporation into the indole alkaloid ajmalicine. The cells used were from the #200 cell line which had previously proven efficacious in tracer studies^{8,15}. As well, for comparison purposes, the mixture of iridodials obtained from the acid promoted cyclization of 10-oxocitronella (from S-(-)-citronellol) was also prepared in deuterated form (at C-1) and used as a substrate. At a dosage of 10 mg/250 ml of cell suspension, ajmalicine (ca. 2-4 mg) was isolated which was specifically labelled with deuterium to the extent of 76% in the case where iridodial derived from nepetalactone was used and 36% in the case where the iridodial mixture obtained by acid promoted cyclization of 10-oxocitronella was used. Even at a dosage of 2.5 mg/250 ml cell suspension, the nepetalactone derived iridodial afforded ajmalicine labelled to the extent of 38%.

In summary, a convenient preparation of iridodial of high isomeric purity was developed and used to corroborate the role of iridodial as an indole alkaloid precursor in a cell suspension of *C. roseus*. The ready availability of iridodial by this means bodes well for further development of the enzymology of this portion of the monoterpene indole alkaloid pathway.

PREPARATION OF IRIDODIAL BY REDUCTION OF NEPETALACTONE - A solution of nepetalactone (110 mg., 0.6 mmol) in tert-butanol (3 ml) was treated with sodium borohydride (14.6 mg, 0.38 mmol). After stirring for 14 h at ambient temperature the reaction mixture was diluted with 10 ml of water and extracted with ethyl acetate (3 x 15 ml). The combined ethyl acetate extract was dried (Na_2SO_4) and concentrated in vacuo. The residue (114 mg) which consisted of a single major and 2 or 3 trace components as indicated by thin layer chromatography, was chromatographed on a silica gel column using hexane-ether (1:1) as eluent to afford pure iridodial (1) (54 mg, 49%) as a colorless film. ^1Hmr (CDCl_3): δ 5.95 (1H, s), 4.80 (1H, d, $J = 5.5$ Hz), 1.50 (3H, s), 1.04 (3H, d, $J = 6.5$ Hz).

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