The Meliacins (Limonoids). Biosynthesis of Nimbolide in the Leaves of *Azadirachta indica*

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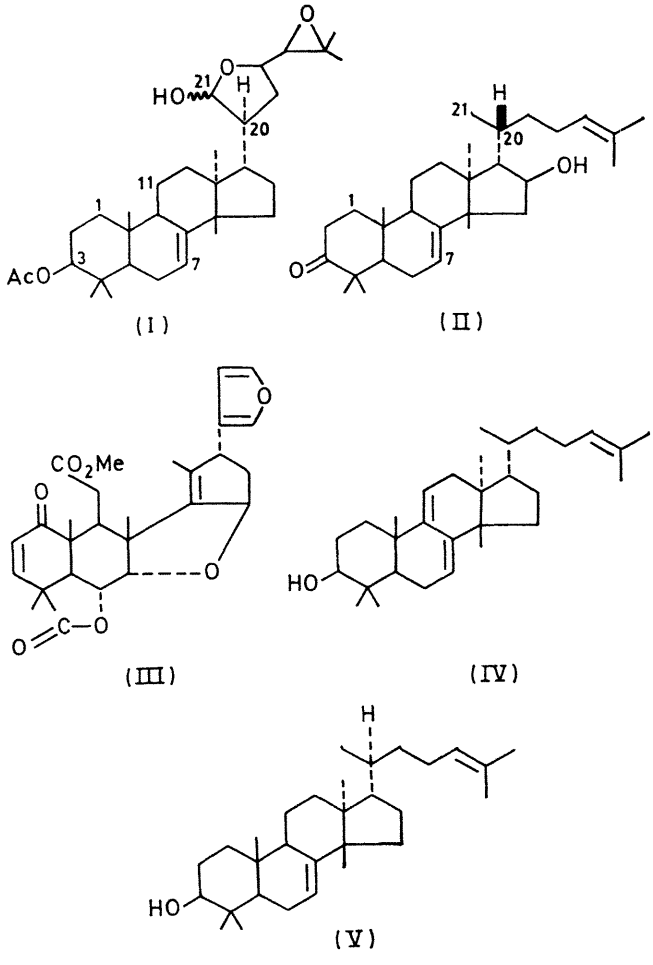
Summary Feeding experiments with the leaves of *Azadirachta indica* have shown that euphol is more efficiently incorporated into the meliacin (limonoid) nimbolide than are its isomers butyrospermol (Δ^7), tirucallol (Δ^8, 20α-H) and Δ^7-tirucallol

AT the time its structural elucidation was reported it was suggested that limonin might arise from an apoeuphol type of triterpene [1] Since then, besides a large number of closely related compounds (meliacins) isolated from the family Meliaceae, many oxygenated C_{30} compounds have been obtained which appear to be intermediates on the suggested biosynthetic route [2] In these presumed protomeliacins notably turraeanthin(I), melianone, aphanamixin (from the family Meliaceae) and flindissol (from *Flindersia dissosperma* family Rutaceae) 20-H has an α-configuration suggesting that the actual precursor is a tirucallol rather than a euphol derivative But because C-21 is an aldehyde in turraeanthin and related compounds there is the possibility of epimerisation at C-20 during biosynthesis Recently another C_{30} compound kulinone (II)[3] in which C 21 is not oxidised to an aldehyde was reported from *Melia azederach* and it was shown to be a euphol derivative

The observation that all known meliacins contain an oxygen function at C 7 has led to the further suggestion that the apoeuphol nucleus found in the meliacins may be formed by rearrangement of a 7α, 8α-epoxide [4] Thus the triterpene precursor of the meliacins may be expected to be a Δ^7 compound In an earlier investigation[5] the terpenoid origin of nimbolide (III) was demonstrated experimentally by the incorporation into it of sodium [2-^{14}C]mevalonate in the leaves of *Azadirachta indica* We have now fed [^{3}H]-euphol [^{3}H]butyrospermol [^{3}H]tirucallol and [^{3}H]-Δ^7-tirucallol to the leaves of the same plant All four triterpenes were incorporated into nimbolide Surprisingly, however [^{3}H]euphol (*i e* Δ^8-isomer) was about 150 times more efficiently incorporated than [^{3}H]butyrospermol (Δ^7 isomer) and about two hundred times more efficiently than [^{3}H]-tirucallol (Δ^8 20α H) (see Table)



TABLE

Precursor	Activity fed (counts/min)[c]	Specific activity of nimbolide (counts/min/mg)	Relative incorporation[d]
[^{3}H]Euphol[a]	67 5 × 10^4	120 6	3 79
[^{3}H]Butyrospermol[a]	61 31 × 10^6	93 4	0 023
[^{3}H]Euphol[b]	41 67 × 10^4	116 4	6 70
[^{3}H]Tirucallol[b]	97 27 × 10^6	117 1	0 0312
[^{3}H] Δ^7 Tirucallol[b]	33 89 × 10^7	77 9	0 0046

[a] Labelled by base catalysed exchange of 2-H with tritiated water followed by borohydride reduction of the 3 ketone
[b] Labelled by reduction of the 3 ketone with sodium borotritide
[c] Counted in a Packard single channel liquid scintillation counter
[d] In view of the difficulty of isolating nimbolide quantitatively from the leaves all the feeding experiments were done on different branches of the same tree at about the same time For the purpose of comparing the efficiency of the use of the different precursors the assumption could therefore be made that the weight of nimbolide was proportional to the weight of leaves used Relative incorporation = (Specific activity of nimbolide × 100 × wt of leaves)/Total activity fed

The triterpenes were labelled by sodium borotritide reduction (or by base-catalysed exchange of 2-H with tritiated water followed by borohydride reduction) of the C-3 ketone They were applied as solutions in methylcellosolve which were painted on the surfaces of the leaves on standing trees The leaves were harvested after 48 h, crushed, and immediately extracted The hot petroleum extract on standing deposited almost pure nimbolide which was filtered off and crystallised to constant activity The petroleum filtrate still contained some nimbolide, but attempts to separate this from other constituents were unsuccessful

Nimbolide, unlike most of the other meliacins, has a seco-ring-c The results of the feeding experiments suggest that its biosynthesis may involve the 7,9(11)-diene (IV) which can easily be formed from an 8α,9α-epoxide † The relative efficiency of incorporation of the triterpene precursors may therefore be different in other meliacins that do not possess a seco-ring-c

Δ^7-Tirucallol (V), which has not hitherto been described in the literature, was prepared from the elemolic acids [6] The mixture of acids was esterified with diazomethane, oxidised to the 3-oxo-derivative and reduced with lithium aluminium hydride to the 3,21-diol Partial mesylation afforded the 21-mesyl derivative which was reduced with $LiAlH_4$ to the 3-hydroxy-compound This was oxidised to the 3-ketone and the Δ^7 and Δ^8 isomers were separated by chromatography on silica gel–silver nitrate to afford 3-oxotirucallone (which did not crystallise) and its Δ^7 isomer which crystallised from acetone and had m p 114—115°, [n m r band at τ 4·7, multiplet (7-H), oxidation with mercuric acetate[7] gave the corresponding 7,9(11)-diene (λ_{max} 235, broad, 223, 247 shoulders)nm]

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† *cf* Conversion of methyl 3α acetoxy-8α,9α-epoxytirucallanoate into the corresponding 7,9(11)-diene by treatment with boron trifluoride etherate in benzene [4]

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