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SYNTHESIS OF DOLICHOLS VIA ASYMMETRIC HYDROGENATION OF PLANT POLYPRENOLS

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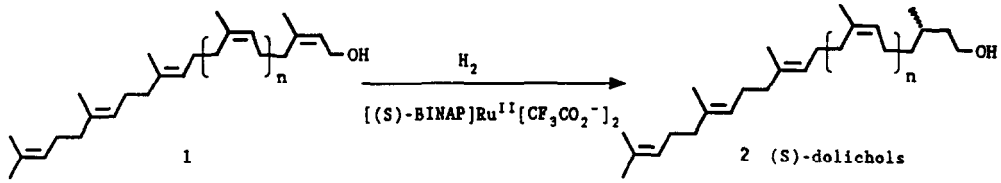
Summary: (S)-Dolichols can be prepared from polyprenols extracted from *Ginkgo biloba* leaves by asymmetric hydrogenation. The dolichols are obtained in high yield and >95% optical purity.

The dihydropolyisoprenols which comprise the homologous family of (S)-dolichols are crucial metabolites in glycoprotein biosynthesis. The hydrophobic nature of the isoprenols plays a key role in anchoring the oligosaccharide moiety at the membrane-bound location where glycoproteins are assembled.[1] In order to study effectively the steps involved in the biosynthesis of lipid-linked oligosaccharides and their subsequent transfer in cotranslational modification processes, it is essential to have a source of pure dolichols in quantities sufficient to allow synthesis of both biosynthetic intermediates and synthetic analogues in the pathways responsible for the biogenesis of glycoproteins. Supplies of naturally occuring dolichols are scarce and can only be isolated from eukaryotic cells in trace quantities by arduous isolation procedures (5kg of pig liver affords approximately 200mg of pure dolichols).[2] Furthermore, the only presently existing synthesis of optically active dolichols from plant polyprenols is lengthy and involves a tedious HPLC resolution step.[3]

A unique and necessary structural feature for biological activity in the dolichols is the single asymmetric center proximal to the terminal hydroxyl group. A recent report by Noyori and coworkers[4] on powerful ruthenium-based homogeneous hydrogenation catalysts for enantioselective hydrogenation of allylic alcohols prompted us to investigate the simple, one-step, conversion of naturally occurring polyprenols into the corresponding dolichols utilizing Δ-bistrifluoroacetato[(S)-2,2'-bis(diphenylphosphino)1,1'-binaphthyl]ruthenium [(S)-BINAP]$Ru^{II}[CF_3CO_2^-]_2$ (*vide infra*).



The starting polyprenols were extracted in the form of the corresponding acetates from mature leaves of the *Ginkgo biloba* tree and saponified as previously described.[5] Polyprenols from this source are reported to have the same alignment of E and Z isoprene units as that of mammalian dolichols and are comprised predominantly of homologues containing 17, 18 and 19 isoprene units (n=13, 14 and 15 compound 1). The α-terminal double bond was assessed to have Z geometry based on ^{13}C NMR spectra of the polyprenols and a variety of model compounds.[6] Prior to saponification, purification of the isolated polyprenyl acetates by both flash silica gel and alumina column chromatography[7] was essential to ensure reasonable substrate to catalyst

ratios in the hydrogenation step, since traces of by-products from the extraction procedure readily poisoned the catalyst. Optimum conditions for the hydrogenation reaction utilizing (S)BINAP-Ru(II) ditrifluoroacetate were as follows: The reaction was carried out in a stainless steel bomb at 25°C and 1500 psi of hydrogen for 1 day, with a 0.35 M solution of the purified polyprenols in dichloromethane/methanol (2:1) as solvent.[8] The substrate to catalyst ratio found to be effective was approximately 100:1 (w/w). This ratio is rather lower than that observed with simpler substrates and may be attributable to the poor solubility properties of the polyprenols in all viable reaction solvents tested. The process however was highly chemoselective and accompanied by less than 2% hydrogenation at any other olefinic site. The dolichols were separated from the catalyst at the end of the reaction by passing the entire mixture through a short silica column.[9] The yield was effectively quantitative.

In determining the optical purity of the product it was important to both assess the degree of stereochemical induction and conclusively prove that the (S)BINAP-Ru(II) catalyst had afforded the desired (predicted) (S)-dolichols: The chirality of the catalyst had been initially selected based on the assumption of Z geometry at the α-terminal double bond. The optical activity was found to be at least 95% in favor of the (S)-isomer.[10] Since the stereochemical course of the hydrogenation is so predictable this result provides furthur evidence confirming the Z geometry of the α-terminal double bond.

Asymmetric hydrogenation of plant polyprenols from the appropriate source represents a viable method for obtaining quantities of both natural (S) and unnatural (R)-dolichols (through use of (R)BINAP-Ru(II) catalyst). Isolation of the polyprenols is facile compared with direct extraction of dolichols[2] and can easily afford multigram quantities due to the high levels (2% dry weight) present in the starting source.

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References and Notes:

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7.The polyprenyl acetates were purified by flash silica gel chromatography eluent 19:1 hexane:ethyl acetate and subsequently applied to an alumina column (Brockman grade 1) equilibrated with 17:3 hexane:ether then eluted with ether.
8.Purified polyprenols are insoluble in methanol, ethanol and ethylene glycol. Of the solvent systems examined 2:1 dichloromethane/methanol gave the best results in the reduction.
9.Eluent 17:3 hexane:ethyl acetate. ^{1}H NMR consistent with J. Burgos, F.W. Hemming, J.F. Pennock, R.A. Morton, *Biochem. J.* (1963), 88, 470. ^{13}C NMR, proton decoupled (75.47MHz, $CDCl_3$) δ: 15.97; 17.64; 23.39; 25.63; 26.41; 26.65; 26.80; 32.00; 32.22; 39.73; 61.20; 124.45; 125.05; 125.44; 134.97; 135.20; 135.33. ^{13}C NMR consistent with polyprenols described by Burgos with the following changes: δ 58.99 shifted to δ 61.20 ($-CH_2OH$), and absence of δ 139.56 ($=CHCH_2OH$).
10.W. Adair and S. Robertson. *Biochem. J.* (1980) 189, 441. The modified method employed to determine enantioselectivity involves oxidation (Jones reagent, acetone) of the resultant dolichols, conversion into the corresponding diastereomeric amides ($SOCl_2$, R-(+)-α-methylbenzylamine) and ozonolysis (O_3,CH_2Cl_2, followed by triphenylphosphine). The resultant product was analysed by HPLC.

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