Tetrahedron Letters,Vol.29,No.29,pp 3603-3606,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

# SYNTHESIS OF 6-EPICASTANOSPERMINE AND 1,6-DIEPICASTANOSPERMINE FROM L-GULONOLACTONE AND SYNTHESIS OF L-6-EPICASTANOSPERMINE AND L-1,6-DIEPICASTANOSPERMINE FROM D-GULONOLACTONE.

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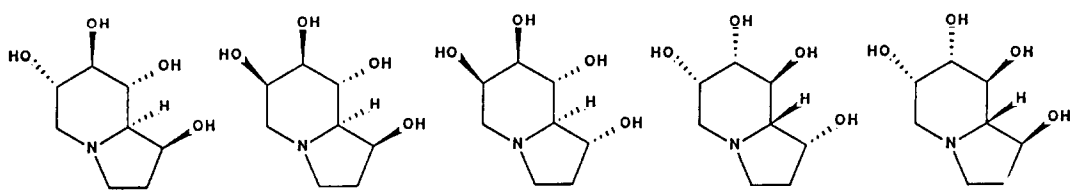
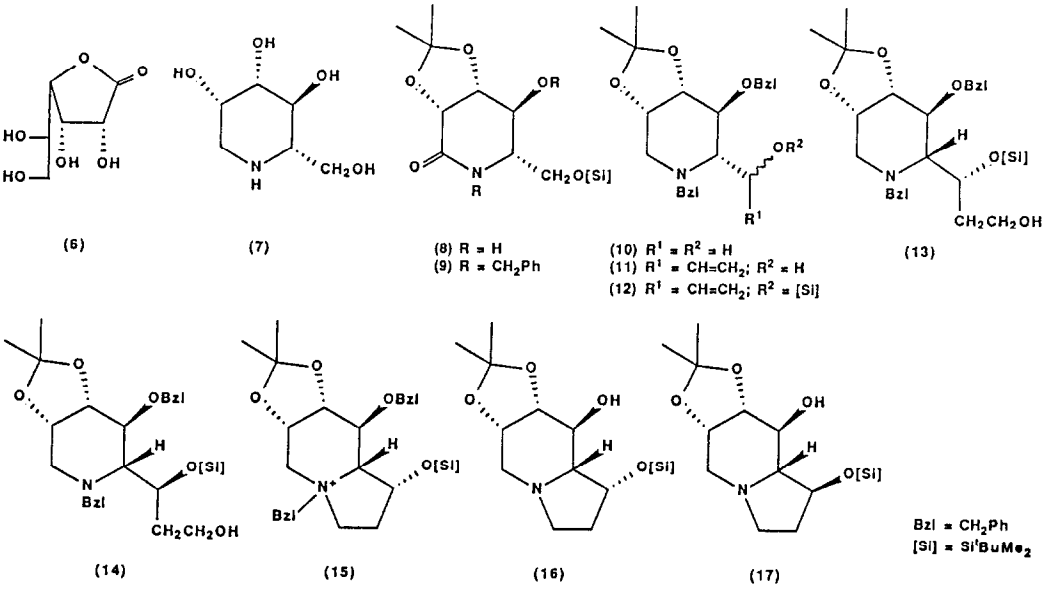
The syntheses of the natural product 6-epicastanospermine [(1S,6R,7R,8R,8aR)-1,6,7,8-tetrahydroxyoctahydroindolizine] and of 1,6-diepicastanospermine [(1R,6R,7R,8R,8aR)-1,6,7,8-tetrahydroxyoctahydroindolizine] from L-gulonolactone and the synthesis of the enantiomers, L-6-epicastanospermine [(1R,6S,7S,8S,8aS)-1,6,7,8-tetrahydroxyoctahydroindolizine] and L-1,6-diepicastanospermine [(1S,6S,7S,8S,8aS)-1,6,7,8-tetrahydroxyoctahydroindolizine] from D-gulonolactone are reported.

Only three polyhydroxylated octahydroindolizines have been isolated from natural sources. Swainsonine, isolated from Astragalus and Swainsona species, is an inhibitor of a mannosidase of glycoprotein processing.[1] Castanospermine (1), which occurs in the seeds of the Australian legume Castanospermum australe,[2] is an inhibitor of several glucosidases[3] including a glucosidase of glycoprotein processing.[4] Castanospermine has been shown to inhibit experimental metastasis in mice[5] and to inhibit human immunodeficiency virus syncytium formation and virus replication;[6] such compounds may have potential as antiretroviral agents.[7] Recently, small amounts of a stereoisomer of castanospermine have been isolated from Castanospermum australe[8] which on the basis of proton and carbon-13 NMR spectroscopy was assigned the structure 6-epicastanospermine (2), epimeric at C-6 with castanospermine. 6-Epicastanospermine (2) is structurally related to deoxymannojirimycin in the same way that castanospermine (1) is related to deoxynojirimycin; the natural product (2) is a powerful inhibitor of amyloglucosidase (an exo-1,4-α-glucosidase) but does not inhibit either α-mannosidase or β-glucosidase.[8] However, a chiral synthesis of (2) indicated that although the spectra and chromatographic properties of (2) were identical to those of the natural product, chiroptical and other properties demonstrated that the natural product has the enantiomeric structure (4).[9] This paper describes the synthesis of 6-epicastanospermine (2) and 1,6-diepicastanospermine (3) from L-gulonolactone and of the respective enantiomers (4) and (5) from D-gulonolactone. 6-Epicastanospermine (2) was shown to be a potent inhibitor of amyloglucosidase whereas the other stereoisomers of castanospermine (3), (4) and (5) did not cause inhibition of this enzyme; this demonstrates that (2), rather than (4), is the correct structure for the natural product.



(1) (2) (3) (4) (5)

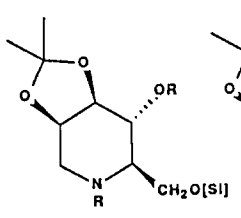
D-Gulonolactone (6) is a suitable starting material for the synthesis of chiral polyhydroxylated pyrrolidines[10] and piperidines, including L-deoxymannojirimycin (7).[11] The protected lactam (8), prepared from (6) in an overall yield of 28% as an intermediate in the synthesis of (7),[11] is also suitable for elaboration to L-6-epicastanospermine (4) and L-1,6-diepicastanospermine (5). Treatment of (8) with benzyl bromide and sodium hydride in tetrahydrofuran in the presence of a catalytic amount of tetrabutylammonium iodide gave the fully protected lactam (9), m.p. 72°-73°C, $[\alpha]_D^{20}$ -53.3° (c, 0.45 in $CHCl_3$), in 95% yield. Reaction of the lactam (9) with lithium aluminium hydride/aluminium trichloride resulted in reduction of the amide function to the corresponding tertiary amine, together with loss of the silyl protecting group, to afford the primary alcohol (10), $[\alpha]_D^{20}$ -6.9° (c, 1.56 in $CHCl_3$), in 76% yield. The structure of (10) was confirmed by removal of the benzyl protecting groups by hydrogenolysis in methanol in the presence of palladium black, followed by hydrolysis with aqueous trifluoroacetic acid, to give L-deoxymannojirimycin (7), identical to an authentic sample.[11]



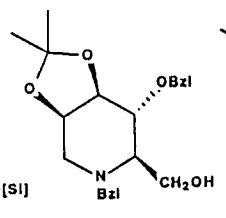

The primary alcohol (10) was subjected to the Swern oxidation [oxalyl chloride, dimethyl sulphoxide, triethylamine, -40°C in dichloromethane] to give the corresponding aldehyde which, on treatment with vinyl magnesium bromide in tetrahydrofuran at room temperature, gave the epimeric alcohols (11) [80% yield]; protection of the allylic alcohols (11) with tert-butyldimethylchlorosilane in dimethylformamide in the presence of imidazole afforded a mixture of diastereomeric silyl ethers (12) in an approximate ratio of 1:1. Hydroboration of the alkenes (12) with borane in tetrahydrofuran, followed by alkaline peroxide oxidation, gave a mixture of diastereomers which could be readily separated by flash chromatography (ethyl acetate:hexane, 1:10) to give the more polar isomer (13), $[\alpha]_D^{20}$ -20.1° (c, 0.83 in $CHCl_3$), in 22% yield and the less polar isomer

(14), $[\alpha]_D^{20}$ -5.1° (c, 0.98 in $CHCl_3$), in 28% yield. The more polar isomer (13) was converted into L-6-epicastanospermine (4). Reaction of (13) with mesyl chloride in the presence of triethylamine in dichloromethane yielded the quaternary ammonium salt (15) [m/z (FAB): 524 (M+, 100%)]; subsequent hydrogenolysis of the benzyl groups in (15) by palladium black in methanol gave (16), $[\alpha]_D^{20}$ +6.5° (c, 0.86 in $CHCl_3$), in 72% yield from (13). Hydrolysis with aqueous trifluoroacetic acid resulted in removal of both the silyl and isopropylidene protecting groups to afford L-6-epicastanospermine (4), $[\alpha]^{24}$ (c, 0.25 in MeOH): -2.5° (589), -2.5° (578), -2.6° (546) -4.8° (436) -11.5° (365), (84% yield); the $^1H$ and $^{13}C$ NMR spectra of (4) were identical to those of an authentic sample of 6-epicastanospermine (2). The less polar isomer (14) was transformed by a similar sequence of reactions into (17), $[\alpha]_D^{20}$ +34.2° (c, 1.62 in $CHCl_3$), in 69% yield; removal of the protecting groups in (17) by hydrolysis with aqueous trifluoroacetic acid gave L-1,6-epicastanospermine (5), $[\alpha]^{24}$ (c, 0.46 in MeOH): +70.0° (589), +73.0° (578), +82.6° (546) +136.7° (436) +208.5° (365), in 85% yield. Both the specific rotations and the NMR spectra[12] of the 1,6-diepi isomer are markedly different from those of the 6-epi isomer.

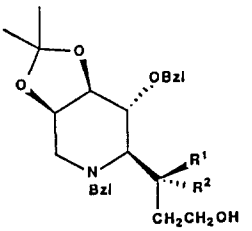
The natural product, 6-epicastanospermine (2), was prepared by an identical sequence of reactions starting from L-gulonolactone. Thus, the lactam (18)[11] was converted to the fully protected benzylated lactam (19), m.p. 72°-73°C, $[\alpha]_D^{20}$ +55.7° (c, 0.55 in $CHCl_3$), in 94% yield which on treatment with lithium aluminum hydride/aluminum chloride gave (20), $[\alpha]_D^{20}$ +8.0° (c, 0.5 in $CHCl_3$), [79% yield]. Further elaboration of (20) by Swern oxidation and treatment with vinyl magnesium bromide gave a mixture of allylic alcohols (80% yield) which were converted to the corresponding silyl ethers and then reacted sequentially with borane:dimethyl sulphide followed by hydrogen peroxide to give the epimeric alcohols (21) and (22) in 60% yield in a ratio of approximately 1:1. The alcohols were separated to give the more polar alcohol (21), $[\alpha]_D^{20}$ +20.8° (c, 0.50 in $CHCl_3$), and the less polar alcohol (22), $[\alpha]_D^{20}$ +3.7° (c, 0.35 in $CHCl_3$). The more polar alcohol (21) was reacted with methane sulphonyl chloride, and then subjected to hydrogenation to give (23) which on hydrolysis with aqueous trifluoroacetic acid gave 6-epicastanospermine (2), $[\alpha]^{24}$ (c, 0.72 in MeOH): +2.0° (589), +2.0° (578), +3.0° (546) +3.5° (436) +10.9° (365) [lit.[8] for (2): $[\alpha]^{24}$ (c, 1.09 in MeOH): +8.0° (589), +8.4° (578), +9.5° (546) +17.4° (436) +13.8° (365)]. Similarly the less polar alcohol (22) was converted into 1,6-diepicastanospermine (3), $[\alpha]^{24}$ (c, 0.72 in MeOH): -72.0° (589), -75.1° (578), -84.8° (546) -139.6° (436) -212.0° (365) respectively.



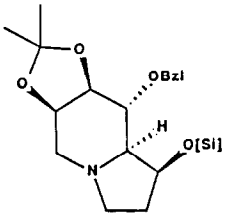

(18) R = H
(19) R = $CH_2Ph$




(20)




(21) $R^1$ = O[Si] ; $R^2$ = H
(22) $R^1$ = H ; $R^2$ = O[Si]




(23)

The optical rotation data obtained for the stereoisomers of castanospermine (2) - (5) supported the original assignment[8] of (2) as the structure of the natural product isolated from Castanospermum australe; however, the specific rotation of 6-epicastanospermine (2) is small and positive where the specific rotation of 1,6-diepicastanospermine (3) is much larger and negative. The natural product (2) was reported to be a potent inhibitor of the enzyme amyloglucosidase, causing 50% inhibition of enzyme activity at about 4 μg/ml.[8] Accordingly, the inhibitory effects of (2) - (5) on the enzymatic activity of amyloglucosidase (EC 3.2.1.3), using p-nitrophenyl-α-D-glucopyranoside as substrate, were evaluated.[13] The assay mixture consisted of 1mM p-nitrophenyl-α-D-glucopyranoside as substrate, 25 mM sodium citrate buffer at pH 5.0, and an appropriate amount of amyloglucosidase all contained in an assay volume of 1 ml. The reaction mixture was incubated at 37°C for 15 min and the reaction was stopped by addition of 2 ml of 0.1 M sodium carbonate, and the generation of p-nitrophenol was determined spectrophotometrically at $OD_{400}$. The effect of the castanospermine isomers on the inhibition of amyloglucosidase was determined by plotting enzyme activity as a function of inhibitor concentration, a fixed amount of enzyme being used throughout, so that 50% inhibition values for the different castanospermine stereoisomers were directly comparable. 6-Epicastanospermine (2) was found to be a strong inhibitor of amyloglucosidase, giving 50% inhibition at 1.4 μg/ml; in contrast, none of the other isomers (3), (4) or (5) showed any significant inhibition of amyloglucosidase activity with less than 10% inhibition at 20μg/ml.

In summary, this paper illustrates the use of the readily available gulonolactones for the synthesis of polyhydroxylated octahydroindolizines and confirms the original[8] structure proposed for 6-epicastanospermine.[14]

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12. $^{13}C$ NMR in $D_2O$ (referenced internally to MeOH) for 6-epicastanospermine (2) and (4): 35.4 (t), 54.6 (t), 58.0 (t), 69.8 (d), 71.5 (d), 72.7 (d), 74.5 (d), 77.9 (d). $^{13}C$ NMR in $D_2O$ for 1,6-diepicastanospermine (3) and (5): 32.3 (t), 51.3 (t), 55.2 (t), 68.8 (d), 71.5 (d), 73.5 (d), 74.0 (d), 75.2 (d).
13. Amyloglucosidase (EC 3.2.1.3) and p-nitrophenyl-α-D-glucopyranoside were purchased from Sigma Chemical Company.
14. Support for this work from SERC (for a postgraduate award to NGR) and from G. D. Searle Monsanto is gratefully acknowledged.

(Received in UK 9 June 1988)