Tetrahedron Letters,Vol.24,No.41,pp 4409-4412,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

THE SYNTHESIS OF 3- and 4-DEUTERATED SHIKIMIC ACID

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Summary: The synthesis of 3-^{2}H- and 4-^{2}H-L-shikimic acids was accomplished starting from commercially available L-shikimic acid.

Shikimic acid[1] is an early-pathway intermediate in the biosynthesis of aromatic compounds in both plants and microorganisms. Since it is stable and generally permeable to cells, it is probably the sole early-pathway molecule that is suitable for tracer-type studies. Floss and co-workers[2], for example, investigated the mechanism of overall conversion of shikimate to chorismate by means of results obtained with 6R- and 6S-^{3}H-shikimic acid molecules which they prepared biosynthetically. Christopherson and Morrison[3], recently prepared 3-^{3}H-shikimic acid, however as an undefined mixture of both 3-^{3}H shikimate and 3-^{3}H-epi-shikimate.

Shikimic acid is a precursor for a divergent array of aromatic compounds. It would be desirable to obtain molecules such as chorismate labeled in the C-3 and C-4 positions in order to pursue, for example, the routes and mechanism of meta-carboxyphenylalanine formation from isochorismate in higher plants[4,5]. Although the enzyme steps of phenylalanine biosynthesis have been established in microorganisms[6], it has not yet been demonstrated in a single plant system. Molecules of chorismate and arogenate[7,8] labeled in the C-3 or C-4 positions would facilitate the study of phenylalanine biosynthesis in higher plants. The first step in achieving this objective is the successful preparation of C-3 and C-4 labeled shikimic acid molecules which may then be converted biosynthetically to molecules such as L-arogenate or isochorismate.

The basic synthetic strategy developed involves the selective protection either of the C-3 and C-5 or C-4 and C-5 alcohol functionalities. An oxidation step followed by reduction with $NaBD_4$ introduces label at the position of the free hydroxyl group. An internally assisted inversion was necessary in the case of the 3-deutero-species. The precise synthetic sequence is detailed below (Fig. 1).

A methanolic solution of shikimic acid 2 was treated with ethereal diazomethane[9], to give methyl-shikimate 3, which was then readily transformed into its corresponding 3,4-orthoacetate 6,[10] without concurrent reaction at the C-5 alcohol. The 5-hydroxyl function was protected as its t-butyldimethylsilyl ether[11], and the orthoacetate moiety hydrolyzed[12], yielding two mono-acetates 1 and 4, each possessing a free, oxidizable C-3 or C-4 hydroxyl group.

Since these two alcohols displayed very similar mobility on thin-layer chromatography, their separation was most easily effected by selectively oxidizing the allylic one using pyridinium

dichromate[13] followed by flash chromatography[14] (petroleum ether : ethyl acetate = 7:1). Unfortunately, the reduction of ketone 5 by $NaBH_4$ (or $NaBD_4$)[15] gave predominantly the thermodynamically more stable β-alcohol 9 (β : α = 90:10). In order to re-establish the correct α-stereochemistry of the hydroxyl group at C-3, alcohol 9 was transformed into its allylic mesylate 10[16]. Solvolysis of this acetoxy-mesylate in methanol containing 1.1 equivalents of potassium acetate at 70°C for 1.5 h readily produced orthoacetate 7 with the desired α-stereochemistry at C-3[17]. Flash chromatography (petroleum ether:ethyl acetate:triethylamine = 120:8:0.2) of the crude product 7, followed by hydrolysis[12] and re-acetylation[18] gave diacetate 11 in 25% yield, (based on ketone 5). Its 200 MHz ^1H nmr spectrum was identical to that of an authentic sample of 11. Furthermore, both mass spectral and 200 MHz ^1H nmr data of deuterated diacetate 11 indicated a quantitative incorporation of the label at C-3. Desilylation at 5°C (0.5 h) using 1.4 equivalents of tetra-n-butylammonium fluoride in dry tetrahydrofuran gave the desired deprotected alcohol as a mixture of acetates in 83% yield, after flash chromatography (petroleum ether:ethyl acetate = 8:7). The deacetylation-demethylation sequence was effected by alkaline hydrolysis in methanol-water[19]. The identity and purity of shikimic acid thus obtained was established by mass spectrometry of its tetra-trimethylsilylated derivative.

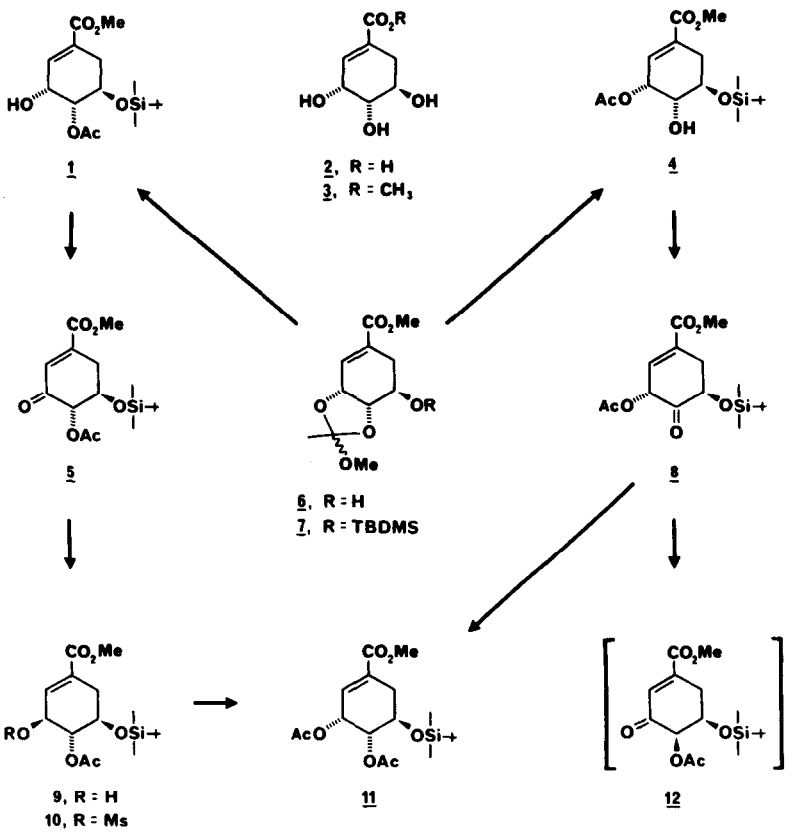

Figure 1

Alcohol 4 proved to be rather inert towards most oxidizing reagents. It was finally transformed to its corresponding ketone 8, albeit in low yield, using Jones reagent[9,20]. Significant amounts of 3-ketone 5 were concurrently produced via acid catalyzed acyl migration. Furthermore, any attempted purification of crude 4-ketone 8, resulted in its almost complete transformation to α, β-unsaturated ketone 12, a product thought to arise via enolization, followed by acyl migration and kinetic protonation. Hence crude ketone 8 was immediately reduced with $NaBH_4$ (or $NaBD_4$)[20], seemingly stereospecifically, to give alcohol 4. The chromato graphic separation of alcohol 4 was facilitated by a prior pyridinium dichromate oxidation[13] of the allylic alcohols; the purified product 4 (obtained in 12% yield based on alcohol 4) was then acetylated in the usual manner[18]. Mass spectral and 200 MHz ^1H nmr data of deuterated diacetate 11 indicated only a 90% incorporation of the label at C-4 (average of 4 runs); this result was attributed to unoxidized starting alcohol 4 which could not be removed prior to reduction. Total deprotection using the same conditions as described for the C-3 deuterated series gave C-4 deuterated shikimic acid.

ACKNOWLEDGMENT:

We thank Dr. George Just for the valuable discussions throughout the proceedings of this work; Dr. Orval Mamer and Ms. Jane Montgomery, The Biomedical Mass Spectrometry Unit, McGill University, for the mass spectra; and Dr. Françoise Sauriol, McGill University, for the 200 MHz ^1H nmr spectra.

Financial support from N.I.H. (to Lolita Zamir) and a post-doctoral fellowship from Université du Quebec, Institut Armand-Frappier (to Corinne Luthe) is gratefully acknowledged.

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10. Methyl shikimate (2.24 g, 1 eq), CH_2Cl_2 (90 ml), trimethyl orthoacetate (3.5 ml, 2.3 eq) p-toluenesulfonic acid (113 mg, 0.05 eq), N_2, rt, 1h; cool to 5°C; add excess Na_2CO_3; extract with ethyl acetate (2 x 600 ml, pre-washed with Na_2CO_3); wash with aqueous Na_2CO_3, then H_2O; 90% yield.
11. E.J. Corey and A. Venkateswarly, J. Am. Chem. Soc., 94, 6190 (1972); alcohol 6 (11.5 mmol), DMF (10 mL), TBDMS Cl (13.9 mmol), imidazole (57.3 mmol), N_2, 45°C, 1 h; extract with ether (2 x 500 mL); wash with H_2O (3 x 100 mL; 4 x 60 mL).
12. Orthoester 7 (3.93 g), MeOH : H_2O : conc. HCl = 90 ml : 30 ml: 3 ml; rt, 1h; add toluene (600 mL); evaporate in vacuo at 35°C; repeat twice.
13. E. J. Corey and G. Schmidt, Tet. Lett. 399 (1979); alcohols 1 and 4 (10.4 mmol), DMF (10 mL), PDC (10.4 mmol), N_2, 45°C, 1 h; add 110 mL H_2O; extract with ether (2 x 600 mL); wash with H_2O (3 x 100 mL, 4 x 70 mL).
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15. Ketone 5 (2 mmol), ETOH (10 mL), $NaBH_4$ (2 mmol), 5°C, 15 min; extract with ether (2 x 600 mL); wash with aq. pH 4 buffer (4 x 100 mL), water (4 x 100 mL); flash chromatography (petroleum ether:ethyl acetate = 7:1).
16. Alcohols 1 and 9 (1.27 mmol), CH_2Cl_2 (10 mL), NEt_3 (7.53 mmol), MSCl (3.88 mmol), N_2, 5°C, 15 min; extract with ether (2 x 175 mL); wash with cold aq. 0.1 N HCl (2 x 25 mL), cold aq. $NaHCO_3$ (3 x 25 mL), H_2O (4 x 25 mL).
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19. Diacetate (117 mg, 1 eq), NaOH (5eq), MeOH (2.5 mL), H_2O (1.5 mL), 5°C/0.5 h; rt/2 h; AM 120 H^+ resin, 15°C; pump off solvents.
20. Alcohol 4 (447 mg), acetone (10 ml), Jones reagent (0.35 ml) rt, 1h; Jones reagent (0.2 ml), 1.5 h; Jones reagent (0.1 ml), 2.5 h; extract with ether (2 x 600 ml); wash with H_2O (5 x 100 ml, 4 x 70 ml). Scaling up not recommended. Reduce with $NaBH_4$ (4 eq, 5°C, EtOH); pour into cold 0.1N HCl (110 ml); extract with ether (2 x 600 ml); wash with cold aq. 0.1N HCl (1 x 100 ml), water (5 x 100 ml).

(Received in USA 20 April 1983)