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# SYNTHESIS OF [RING B-$^{13}C_6$] DIOSMIN

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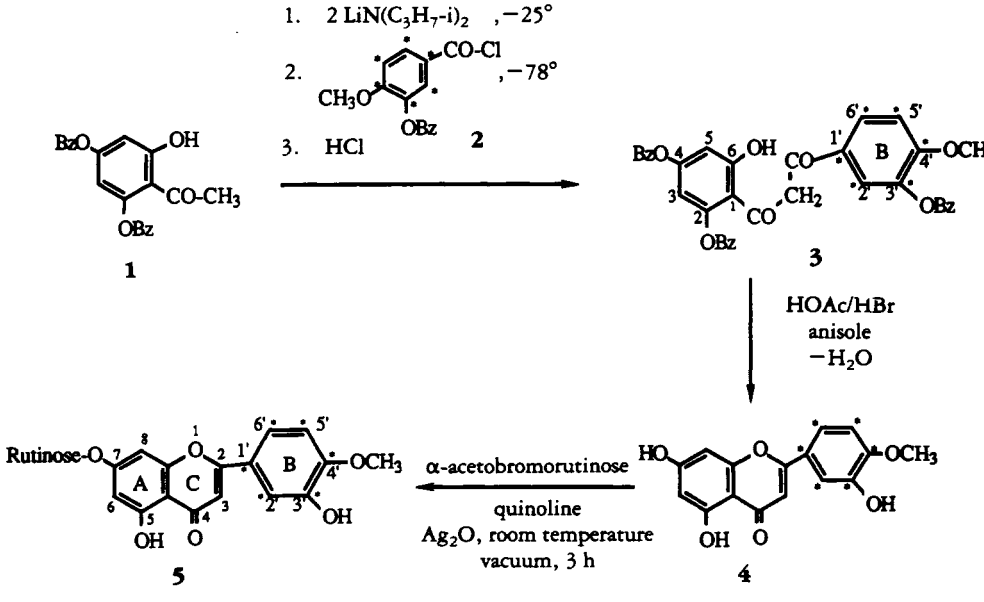
ABSTRACT.—End products of flavone metabolism are phenolic acids, originating from rings B and $C_2$ and/or $C_3$ of ring C. Several of them are also endogenous products. Synthesis of uniformly labeled ring B-$^{13}$C-diosmin [**5**] was performed for the unambigous distinction, by ms, between endogenous and exogenous phenolic acids.

In connection with our work on the metabolism of diosmin [**5**] in humans, we required a supply of the flavonoid diosmin containing a uniformly $^{13}$C-labeled B-ring. Of the various synthetic methods available for flavones (1–13), the method of Barneji and Goomer (13) gave the best yield of the methods that were tested, and this was selected for the synthesis.

The synthetic pathway is shown in Scheme 1; the labeling at each step was checked by determining the mass spectra of the labeled products, which differed by 6 mass units from those of the corresponding unlabeled compound. The preparation of the protected phloroacetophenone **1** was effected according to the method of Honohan *et al.* (14), replacing hexamethylphosphoramide with DMF. The coupling of **4** with rutinose was made using the Königs-Knorr reaction (15), previously applied by Zemplén and Bognar (16) to the synthesis of hesperidin; this is still the best way of carrying out this conversion. The product **5** was obtained in 0.6% yield from the labeled precursor **2**.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Authentic diosmin and diosmetin were supplied by SERVIER Laboratories (France). The nmr measurements were recorded at 360 MHz in DMSO-$d_6$ (Brucker WM 360 WB). The glc system (DI 300-Delsi) employed a SE-30 capillary column (25 m × 0.2 mm i.d.) and temperature programming from 60° to 280°; the injector system was used in the splitless mode at 260°. The mass spectrometer (R 10-10 C-Nermag) em-




SCHEME 1

ployed an ionization-source temperature of 280° and ionization energy of 70 eV; the mass spectra were scanned from 20 to 600 amu. Hplc analyses of diosmin have been carried out on a Waters System incorporating a μ-Bondapak $C_{18}$ column and a M440 uv-detector fixed at 254 nm; the samples, diluted in DMSO-MeOH (45:5), were eluted with MeCN-$H_2O$-HOAc (25:65:10).

DIBENZYLPHLOROACETOPHENONE [**1**].—Phloroacetophenone (1.7 g, 0.010 mol), dissolved in DMF (25 ml) in the presence of anhydrous $K_2CO_3$ (0.828 g, 0.006 mol), was treated with benzyl chloride (0.756 g, 0.006 mol), and the solution was stirred under argon at room temperature in the dark for 8 days. The suspension was then filtered off and the filtrate acidified with HOAc (pH 4) and concentrated under vacuum. The residual crude product was taken up with EtOAc, washed with $H_2O$, a solution of KOH (5%), $H_2O$, and saturated NaCl, and then dried over $Na_2SO_4$. After removal of the solvent, the residue was chromatographed on a Si gel column; the product **1** was eluted with EtOAc-hexane (20:80) in 30% yield, mp 112–115° [lit. (17) mp 90–100° (MeOH) working with $K_2CO_3$ in $Me_2CO$; we were unable to duplicate this result in that way].

[RING-$^{13}C_6$]-*O*-BENZYLISOVANILLOYL CHLORIDE [**2**].—[Ring-$^{13}C_6$] isovanillic acid was obtained from Cambridge Isotope Laboratories (USA) [$^{13}C$ atom % was 99% (ms, $^{13}C$ nmr)] and was used undiluted.

This isovanillic acid (17 g, 0.1 mol) was suspended in MeOH (250 ml), and a stream of HCl was passed through the refluxing solution at 70°; dissolution was complete in 4 h. The solvent was removed under vacuum to yield 16.5 g of methyl [ring-$^{13}C_6$] isovanillate (90%): mp 64–66° [lit. (10) 65–66°].

This ester (11 g, 0.060 mol) was dissolved in MeOH (100 ml) in the presence of anhydrous $Na_2CO_3$ and benzyl chloride (10 ml, 0.1 mol), and the solution was refluxed for 8 h. After cooling filtration and washing with $H_2O$, the residue was recrystallized from an $Me_2CO$/MeOH mixture: yield 12.5 g (77%); mp 120–124° [lit. (10) 124°]. The benzylated ester was dissolved in a 1 M solution of KOH (170 ml) and refluxed for 2 h. After cooling, the clear solution was acidified with 10% $H_2SO_4$, and the precipitated product was filtered, washed with $H_2O$, and dried: yield 9.3 g (78%); mp 175–177° [lit. (10) 177°].

The previously obtained acid (7 g, 0.027 mol) was dissolved in anhydrous $Et_2O$ (200 ml), and thionyl chloride (10 ml) was slowly added, together with a few drops of pyridine. The mixture was refluxed for 12 h, then concentrated under vacuum. The crystallized product, mp 79–80°, showed a positive reaction with alcoholic $AgNO_3$ and gave the corresponding amide by action of $NH_3$ in $C_6H_6$: yield 6.6 g (89%).

[RING B-$^{13}C_6$]-3′,4,6-TRIBENZYLOXY-DIBENZOYLMETHANE [**3**].—A solution of dibenzylphloroacetophenone (4.8 g, 0.0137 mol) in pure THF (10 ml) was added to a stirred solution of lithium diisopropylamide (10 mM, from diisopropylamide and butyllithium) (18) in THF at −25°. The mixture was stirred, then cooled to −78°, and [ring-$^{13}C_6$]-*O*-benzylisovanilloyl chloride (5 g, 0.018 mol) in THF was added. The mixture turned yellow soon after the addition of the aryl chloride. Stirring was continued at −78° for 3 h, and the mixture was allowed to warm up to room temperature and set aside overnight. It was then diluted with EtOAc and acidified to pH 3 with dilute HCl. The organic layer was dried with $Na_2SO_4$, and the solvents were removed to give the β-diketone as a crystalline solid. The product was recrystallized from EtOAc/pentane to give the product **3**: yield 2.8 g (35%); mp 157–160°. The structure was confirmed by $^1H$-nmr spectrometry (19): 3.85 ppm (3H, OMe), 4.55 ppm (1.2 H, CO-$CH_2$-CO corresponding to the keto form), 4.90 ppm (2H, $CH_2$-Ph), 5.12 ppm (2H, $CH_2$-Ph), 5.18 ppm (2H, $CH_2$-Ph), 6.15–6.40 ppm (2H, H-3 and H-5), 6.95 ppm (1H, H-5′), 7.18 ppm (0.8 H, =CH, corresponding to the enol form), 7.30–7.40 ppm (15 H, aromatic rings), 7.40–7.55 ppm (2H, H-2′ and H-6′), 12.5 ppm (1H, OH), 13.6 ppm (1H, OH). Comparing the intensities of the 7.18 ppm and 4.55 ppm peaks, it is concluded that the enol form predominates.

[RING B-$^{13}C_6$]-DIOSMETIN [**4**].—The β-diketone (5 g, 0.0085 mol) was dissolved under argon in glacial HOAc (80 ml), and anisole (8 ml) and 48% HBr in HOAc (10 ml) were added. The mixture was stirred and heated at 90° until complete dissolution; the mixture was then set aside overnight at 70°. It was then poured onto ice, and anisole and benzyl bromide were removed by steam distillation; the residue was filtered, dissolved in $Me_2CO$, and precipitated by $H_2O$ to yield 2.14 g of crude product (84%). This product (2.14 g) was dissolved in $Ac_2O$ (6 ml) with pyridine (4 ml). The solution was heated at 70° for 1 h, then poured onto ice and the resultant white precipitate isolated by suction, dried, and recrystallized from absolute EtOH to yield 1.80 g (42%) of [ring B-$^{13}C_6$]-diosmetin triacetate, mp 192–194° [lit. (20) 193–194°]. The triacetate (1.80 g) was dissolved in alcohol (10 ml) at 90°, and a 3% solution of NaOH (2 ml) was added. The yellow solution was heated at 60° for 15 min, then diluted with $H_2O$ (10 ml), and finally acidified after cooling with 10% $H_2SO_4$ (pH 2). After 24 h at 0°, the product was filtered, dried, and recrystallized from EtOH: mp 250–255° [lit.

(20) 250–255°]; yield 0.888 g (70%). $^1$H-nmr (DMSO-$d_6$) 3.9 ppm (s, OMe), 6.17 ppm (d, H-6), 6.45 ppm (d, H-8), 6.72 ppm (s, H-3), 7.15 ppm (d, H-5′), 7.4–7.6 ppm (cluster, H-2′ and H-6′) [lit. (21)].

The product was methylated with $CH_2N_2$ in $Et_2O$ for gc-ms analysis and compared with authentic methylated diosmetin. The mass spectra were identical except that the fragments differed by 6 mass units: *m/z* 334 $[M]^+$, 305 (100) for compound **4**; *m/z* 328 $[M]^+$, 299 (100) for authentic unlabeled methylated diosmetin.

[RING B-$^{13}C_6$]-DIOSMIN [**5**].—[Ring B-$^{13}C_6$]-diosmetin (0.200 g, 0.00066 mol) was mixed with acetobromorutinose (0.440 g, 0.0011 mol) (22) with a glass rod by trituration, and freshly distilled quinoline (2 ml) and active $Ag_2O$ (0.140 g) were added. After a short stirring, the mixture was left under vacuum over $H_2SO_4$ for 3 h. HOAc (30 ml) was then added to the mixture, the mixture was centrifuged, and the supernatant was poured into $H_2O$ (300 ml). After 12 h, the product was filtered, washed with $H_2O$, dried under vacuum in the presence of $H_2SO_4$, taken up with $CHCl_3$ and filtered; the residue was finally dissolved in EtOH. After 24 h, a dark powder was filtered off, yield 0.100 g (17%). The crude product was dissolved in $Ac_2O$ (5 ml), and pyridine (3 ml) was added. The solution was heated at 70° for 2 h and checked by tlc [EtOAc-$CH_2Cl_2$ (30:70); the spot is blue under uv]. Ice was added and the resultant precipitate isolated by suction. The product was chromatographed on a Si gel column with EtOAc-$CH_2Cl_2$ (50:50). The pure fractions were pooled, evaporated under vacuum, and recrystallized from hot EtOH. After cooling for 12 h, a white product was obtained, with mp 140–150° [lit. (20) 146–150°], exactly similar to authentic diosmin octaacetate prepared for comparison: yield 0.080 g (74%). The diosmin octaacetate was deacetylated with 1 N NaOH (5 ml) in EtOH (5 ml) for 1 h at 70°. After cooling, the mixture was diluted with $H_2O$ and acidified by HOAc. The yellow solution was cooled at 0°, and after 24 h the precipitated product was isolated by suction; hplc and uv analyses were identical with authentic diosmin: yield 0.050 g (97%). $^1$H-nmr (DMSO-$d_6$) 1 ppm (3H, m, Me-rhamnose), 3.2–4 ppm (10 H, m, glucose and rhamnose), 3.9 ppm (3H, s, OMe), 4.2 ppm (m, H-1 rhamnose), 5 ppm (m, H-1 glucose), 6.48 ppm (d, H-6), 6.75 ppm (d, H-8), 6.82 ppm (s, H-3), 7.45 ppm (d, H-2′), 7.57 ppm (dd, H-6′), 7.15 ppm (d, H-5′). The chemical shifts of the signals in the $^{13}$C-nmr spectrum were identical to the literature values (23).

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