Synthesis of the [^{2}H]-Labelled Urinary Lignans Enterolactone and Enterodiol

David N. Kirk *,† **and Leo M. McLaughlin**
Department of Chemistry, Westfield College, University of London, Kidderpore Avenue, Hampstead, London NW3 7ST
Alexander M. Lawson, Kenneth D. R. Setchell, and Shailesh K. Patel
Section of Clinical Mass Spectrometry, Clinical Research Centre, Harrow HA1 3UJ

Isotopically labelled forms of the urinary lignans (±)-*trans*-2,3-bis-(3-hydroxybenzyl)butan-4-olide (enterolactone) and (±)-2,3-bis-(3-hydroxybenzyl)butane-1,4-diol (enterodiol) have been synthesized from [4,4-$^2H_2$]butenolide, obtained from the furan–maleic anhydride adduct by reduction ($NaBD_4$) and pyrolysis.

Two novel lignans of the dibenzylbutane class,[1] unique in lacking *para*-substitution in the benzylic groups, have recently been found in human and animal urines.[2–4] Immediate interest was aroused by the observation of distinctive excretory patterns in the female during ovulatory cycles and pregnancy. These lignans, subsequently found in other body fluids,[5,6] are now known to have a dietary origin,[5,7–12] one source at least being a diglycoside (**1**) of 2,3-bis-(4-hydroxy-3-methoxybenzyl)butane-1,4-diol (secoisolariciresinol) which is particularly abundant in linseed,[12] and probably occurs in other plant products. Intestinal bacteria are responsible[5,12] for the structural changes, including deoxygenation at the *para*-positions, and oxidation of the butane-1,4-diol system to form the γ-butyrolactone, which lead to the urinary lignans (±)-2,3-bis-3-(hydroxybenzyl)-butane-1,4-diol (enterodiol)[7] (**2a**) and (±)-*trans*-2,3-bis-(3-hydroxybenzyl)butanolide (enterolactone)[7] (**3a**). Surprisingly the natural urinary lignans are racemic.[2,13]

The physiological significance of these lignans, and of their pattern of excretion, is still a subject for speculation. To aid further study, and to permit accurate and rapid mass spectrometric estimation of the lignans in samples of biological origin, we have synthesized both lignans in [9,9-$^2H_2$]-labelled form, and also [9,9,9′,9′-$^2H_4$]enterodiol, for use in isotope-dilution measurements with selected-ion monitoring.[14]

Several syntheses of enterolactone have been described.[13,15,16–19] Our own[15] original synthesis *via* a double Stobbe condensation provided (±)-*trans*-2,3-bis-(3-acetoxybenzyl)succinic anhydride (**4**) as a key intermediate which was reduced by sodium borohydride to give the corresponding butanolide (**5**). Substitution of [$^2H_4$]borohydride afforded the first sample of [9,9-$^2H_2$]enterolactone (**3b**), but the inefficiency of the Stobbe method prompted us to seek a more satisfactory synthesis.

It seemed to us that the tandem conjugate addition, used by Ziegler[20] in a synthesis of the stegane skeleton, could be applied to this end. We were actively pursuing this approach when Pelter,[16] reported he had successfully utilized this method in the synthesis of enterolactone and enterodiol and had obtained high yields. Following the authors'[16] suggestion we had no difficulty in repeating this procedure with benzyl rather than methyl protecting groups at the phenolic positions.

Thus Michael addition of the anion of an arylbis(phenylthio)methane (**6**) to butenolide (**7a**), followed by trapping of the anionic intermediate with a benzyl bromide, gave the protected lignan (**8a**) in excellent yield. Desulphurisation of (**8a**) with Raney nickel gave enterolactone (**3a**) in quantitative yield. This approach has the inherent advantage that the benzyl groups are removed concomitantly with the phenylthio groups thus eliminating a synthetic step.

In order to modify the Pelter synthesis to produce the [9,9-$^2H_2$]lignans, we needed [4,4-$^2H_2$]butenolide (**7b**) as the starting material. This compound was readily prepared from the Diels–Alder adduct (**9**) of furan and maleic anhydride. Reduction of the anhydride with sodium [$^2H_4$]borohydride gave the bicyclic lactone (**10**), which was efficiently cleaved by pyrolysis to give [4,4-$^2H_2$]butenolide. The corresponding process with unlabelled borohydride proved to be a particularly convenient route to butenolide itself. The 'tandem' benzylation process proceeded smoothly, with no loss of deuterium from the labelled butenolide, to give [9,9-$^2H_2$]enterolactone (**3b**) after hydrogenolytic removal of the benzyl protecting groups.

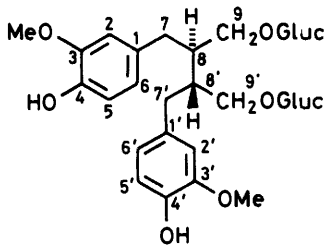
[^{2}H]-Labelled enterodiol was then readily available in two forms: [9,9-$^2H_2$] — either by reducing unlabelled enterolactone (**3a**) with $LiAlD_4$ or by reducing [9,9-$^2H_2$]enterolactone (**3b**) with $LiAlH_4$, and [9,9,9′9′-$^2H_4$] — by reducing the labelled lactone (**3b**) with $LiAlD_4$.

Experimental

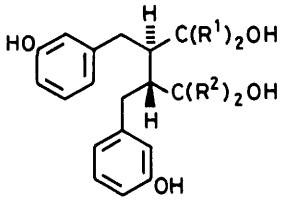
M.p.s were determined with a Reichert hot-stage apparatus. I.r. spectra refer to KBr discs unless otherwise indicated. N.m.r. spectra were determined at 100 MHz for solutions in deuteriochloroform with tetramethylsilane as internal standard. All solvents were distilled before use. Light petroleum refers to the fraction, b.p. 60—80 °C. Tetrahydrofuran (THF) was dried by distillation from sodium.

(±)-trans-2-(3-*Benzyloxybenzyl*)-3-[3-*benzyloxy*-α,α-*bis*-(*phenylthio*)*benzyl*]*butyrolactone* (**8a**).—3-Benzyloxybenzaldehyde bis(phenylthio) acetal (**6**) (4.14 g, 10 mmol) was dissolved in anhydrous THF under argon and the solution was cooled to −78 °C. This solution was treated with n-butyllithium (11 mmol) and the mixture was stirred at −78 °C for 40 min, then butenolide (**7a**) (0.86 g, 10 mmol) was added slowly to the mixture which became yellow in colour. The reaction mixture was stirred for 30 min at −78 °C and was then treated dropwise with a solution of 3-benzyloxybenzyl bromide (2.77 g, 10 mmol) in dry THF (5 ml), followed 1 h later by tetramethylethylenediamine (3.23 g, 10 mmol). The mixture was allowed to warm slowly to room temperature overnight, then water (100 ml) was added. The mixture was extracted with dichloromethane and after being washed with water the extracts were dried over magnesium sulphate. Removal of solvent left a brown gum (6 g) which was chromatographed on silica gel using gradient elution with light petroleum–ethyl acetate to give the product (**8a**) as a gum (4.5 g, 65%) which appeared to be homogeneous (t.l.c.), and the n.m.r. spectrum indicated only

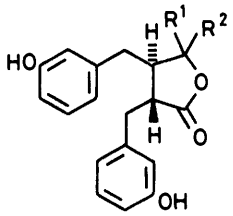
† *Present address*: Department of Chemistry, Queen Mary College, Mile End Road, London E1 4NS.



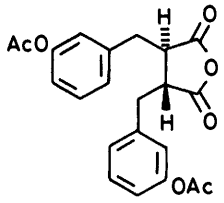
(1)



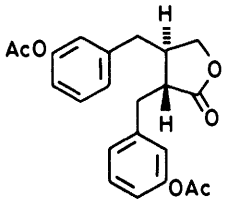
(2) a; $R^1 = R^2 = H$
b; $R^1 = H, R^2 = D$
c; $R^1 = R^2 = D$



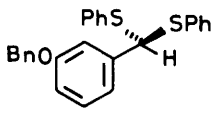
(3) a; $R^1 = R^2 = H$
b; $R^1 = R^2 = D$



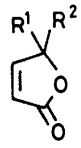
(4)



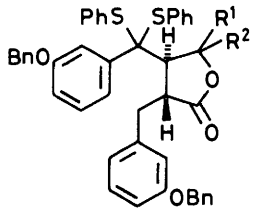
(5)



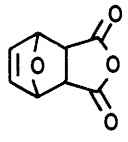
(6)



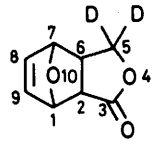
(7) a; $R^1 = R^2 = H$
b; $R^1 = R^2 = D$



(8) a; $R^1 = R^2 = H$
b; $R^1 = R^2 = D$



(9)



(10)

Bn = benzyl
Gluc = glycoside

trace contaminants (< 5%); $\nu_{max.}$($CHCl_3$) 1 780 and 1 660 cm^{-1}; δ_H* 2.6—3.6 (5 H, m, 7'a-, 7'b-, 8-, 8'-, and 9a-H), 4.4 (1 H, dd, *J* 10 and 3 Hz, 9b-H), 4.94 (4 H, s, 2 × $PhCH_2$), and 6.4—7.4 (28 H, m, ArH); *m/z* 585 (M^+ − SPh, 3%), 475 (4), 305 (4), 181 (3), and 91 (100) [Found: $(M - SPh)^+$, 585.2115. $C_{44}H_{38}O_4S_2$ requires *M* − SPh, 585.209 96]. This material was used without further purification for the following stage.

(±)-trans-2,3-*Bis*-(3-*hydroxybenzyl*)*butyrolactone* (*Enterolactone*) (**3a**).—The conjugate addition product (**8a**) (700 mg, 1 mmol) was dissolved in THF (4 ml) and absolute ethanol (25 ml) was added. Raney nickel (4 g) was added to this solution and the mixture was stirred under reflux for 5 h. Removal of the Raney nickel and solvent and chromatography of the product on silica gel using gradient elution with light petroleum–ethyl acetate gave a clear gum (300 mg, 100%). Crystallization from dichloromethane gave enterolactone (**3a**) as a solid (75%), m.p. 142—143 °C (lit.,[13] 141—143 °C), with spectra identical with those reported previously:[13,15,16-19] δ_H† 2.52 (4 H, m, $w_{\frac{1}{2}}$ 7 Hz) and 2.92 (2 H, m, $w_{\frac{1}{2}}$ 9 Hz) (together $ArCH_2CHCHCH_2Ar$), 3.87 and 4.13 (2 H, dd of doublets, *J* 10 and 6 Hz, CH CH_2O), 5.35 (br s, $w_{\frac{1}{2}}$ *ca.* 50 Hz, OH) 6.48—6.76 (6 H, complex, ArH), and 7.06—7.25 (2 H, dt, ArH); $\nu_{max.}$(KBr) 3 380 (OH) and 1 747 cm^{-1} (lactone); *m/z* [as bis(trimethylsilyl ether)] 442 (M^+) and 180 (base peak).

[5,5-$^2H_2$]-4,10-*Dioxatricyclo*[5.2.1.$0^{2,6}$]*dec*-8-*en*-3-*one* (**10**).—To a stirred suspension of $NaBD_4$ (2.5 g, 59.5 mmol) in dry dimethylformamide (DMF) (50 ml) cooled in an ice-bath was added in small portions the anhydride (**9**). The ice-bath was removed and the mixture was stirred for a further 1 h. 6M-Hydrochloric acid (20 ml) was added and the mixture was concentrated under reduced pressure. Water (200 ml) was added and the product was extracted with chloroform. The extract was washed successively with water, 5% aqueous sodium hydrogen carbonate, and again with water, and dried over anhydrous magnesium sulphate. Removal of solvent left a clear liquid (mainly DMF) which, when subjected to high vacuum, gave a solid. This product was purified by chromatography on silica gel using gradient elution with light petroleum–ethyl acetate. The *lactone* (**10**) crystallized from benzene as needles (4.47 g, 69%), m.p. 95—96 °C (lit.,[21] 98 °C for non-deuteriated material); $\nu_{max.}$(Nujol) 1 750 cm^{-1} (C=O); δ_H 2.8 (2 H, m, 2- and 6-H), 4.96 (1 H, m, 7-H), 5.25 (1 H, m, 1-H), and 6.44 (2 H, m, 8- and 9-H); δ_C 175.3 (C=O), 136.2 (C-8 and -9), 83.8 (C-1), 81.6 (C-7), 47.7 (C-2), and 41.3 p.p.m. (C-6); *m/z* 154 (M^+, 0.1%), 125 (M^+ − CHO, 1), 68 ($C_4H_4O^+$, 67), and 39 ($C_3H_3^+$, 100) (98% $^2H_2$).

[4,4-$^2H_2$]*Butenolide* (**7b**).—The lactone (**10**) (2.0 g, 13 mmol) was placed in a Kugelrohr bulb-to-bulb distillation apparatus and the system was evacuated to 16 mmHg pressure. The temperature was slowly increased to 200 °C, when there was a rapid evolution of furan. The product was distilled (bulb-to-bulb) to give [4,4-$^2H_2$]butenolide (**7b**) (0.97 g, 87%) which had i.r. and ^1H n.m.r. data similar to those reported,[22] and was shown to be homogenous on t.l.c.; $\nu_{max.}$ 2 205, 2 100, and 1 782—1 766 cm^{-1} (CO) (lit.,[22] values identical); δ_H 6.18 (1 H, d, *J* 5.8 Hz) and 7.61 (1 H, d, *J* 5.8 Hz) [lit.,[22] δ (CCl_4) 5.90 (1 H, d, *J* 5.4 Hz) and 7.45 (1 H, d, *J* 5.4 Hz)].

(±)-trans-[4,4-$^2H_2$]-2-(3-*Benzyloxybenzyl*)-3-[3-*benzyloxy*-α,α-*bis*(*phenylthio*)*benzyl*]*butyrolactone* (**8b**).—Prepared in the same manner as (**8a**), except that 3-benzyloxybenzyl iodide was used instead of the bromide, the product was obtained as a clear *oil* (6.3 g, 90%) after chromatography on silica gel, $\nu_{max.}$($CHCl_3$) 1 780 and 1 660 cm^{-1}; δ_H* 2.6—3.4 (4 H, m, 7'a-, 7'b-, 8-, and 8'-

* Numbering scheme as for compound (**1**).

† For a high-field (400 MHz) n.m.r. analysis, and complete ^1H and ^{13}C assignments, see G. Cooley, R. D. Farrant, D. N. Kirk, S. Patel, S. Wynn, M. J. Buckingham, G. E. Hawkes, M. B. Hursthouse, A. M. R. Galas, A. M. Lawson, and K. D. R. Setchell, *J. Chem. Soc., Perkin Trans. 2*, 1984, 489.

H), 4.96 (4 H, s, 2 × PhCH_2), and 6.4—7.4 (28 H, m, ArH); *m/z* 587 (4%), 478 (4), 388 (3), 305 (18), and 91 (100) [Found: (*M* − SPh)$^+$, 587.222 91. $C_{44}H_{36}{}^2H_2O_4S_2$ requires (*M* − SPh), 587.222 534].

[4,4-$^2H_2$]-(±)-trans-2,3-*Bis*-(3-*hydroxybenzyl*)*butyrolactone* ([9,9-$^2H_2$]*Enterolactone*) (**3b**).—The protected lignan (**8b**) (130 mg, 0.2 mmol) was dissolved in THF (4 ml) and absolute ethanol (25 ml) was added. Raney nickel (2 g) was added to this solution and the mixture was stirred under reflux for 5 h. Removal of the Raney nickel and solvent and chromatography of the product on silica gel using gradient elution with light petroleum–ethyl acetate gave [9,9-$^2H_2$]*enterolactone* (**3b**) in quantitative yield. The product was essentially identical (m.p., t.l.c., ^{1}H n.m.r.) with a sample prepared by our previously described route[15] *via* the Stobbe reaction (but with use of NaB^2H_4 instead of $NaBH_4$ to reduce the intermediate succinic anhydride derivative to the corresponding butenolide), except that the n.m.r. signals at δ_H* *ca.* 3.8 and 4.1 (9-H_2) were absent for the deuteriated sample; *m/z* [as bis(trimethylsilyl ether)] 444 (*M*$^+$, 12%), 265 (6), 207 (12), 181 (91), and 180 (100) (94% $^2H_2$) (Found: *M*$^+$, 300.132 416. $C_8H_{16}{}^2H_2O_4$ requires *M*, 300.133 064).

[1,1-$^2H_2$]-(±)-2,3-*Bis*-(3-*hydroxybenzyl*)*butane*-1,4-*diol* ([9′,9′-$^2H_2$]*Enterodiol*) (**2b**).—$LiAlD_4$ (136 mg, 3.7 mmol) was suspended in dry THF (20 ml) and a solution of enterolactone (**3a**) (156 mg, 0.5 mmol) in THF (3 ml) was added cautiously. The mixture was stirred at room temperature for 1 h and then boiled under reflux for a further 2.5 h. The excess of $LiAlD_4$ was destroyed with water and the mixture was then poured into a mixture of very dilute hydrochloric acid and ether. More hydrochloric acid was added until the precipitate disappeared and the mixture was then extracted with ether, and the extract was washed with water until neutral and dried over magnesium sulphate. Removal of solvent gave a clear oil. This was taken up in a small amount of ethyl acetate and dichloromethane was added. The mixture was kept in a refrigerator and the [9′,9′-$^2H_2$]*enterodiol* (**2b**) crystallized out as a solid (150 mg, 94%), m.p. 177—179 °C (lit.,[18] 175—176 °C for non-deuteriated material); $\nu_{max.}$(KBr) 3 410, 3 150br sh, and 1 588 cm^{-1}; δ_H($CDCl_3$–C_5D_5N) 1.96 (2 H, br s, CHCH), 2.64—2.85 (4 H, m, 2 × ArCH_2), 3.5 (dd, *J* 11.5 and 4.2 Hz) and 3.8 (dd, *J* 11.5 and 2.0 Hz) (together CH_2OH), 5.8 (2 H, br s, aliphatic OH), 6.63—6.8 (6 H, m, ArH), and 7.0—7.2 (2 H, m, ArH); δ_C*($CDCl_3$–C_5D_5N) 157.1 (C-3 and -3′), 142.1 (C-1 and -1′), 128.6 (C-5 and -5′), 119.5 (C-6 and -6′), 115.9 (C-2 and C-2′), 112.6 (C-4 and -4′), 59.4 (C-9), 43.7 (C-8 or 8′), 43.6 (C-8′ or -8), and 35.9 p.p.m. (C-7 and -7′); *m/z* [as tetrakis(trimethylsilyl ether)] 502(*M*$^+$ − Me_3SiOH), 6%), 412(*M*$^+$ − 2 × Me_3SiOH, 66), 233 (48), 181 (66), and 180 (100) (98% $^2H_2$) (Found: *M*$^+$, 304.163 850. $C_{18}H_{20}{}^2H_2O_4$ requires *M*, 304.164 364).

[1,1,4,4-$^2H_4$]-(±)-2,3-*Bis*-(3-*hydroxybenzyl*)*butane*-1,4-*diol* (**2c**).—Prepared by reduction of [9,9-$^2H_2$]enterolactone (**3b**) with $LiAlD_4$ by the method described above, this compound was obtained as a solid in 90% yield, m.p. 177—179 °C (lit.,[18] 175—176 °C for non-deuteriated material); *m/z* 306 (*M*$^+$, 1%), 288 (5), 270 (3), 181 (10), 162 (14), 135 (14), 109 (100), 108 (74), 107 (64), and 77 (18) (97% $^2H_2$).

References

1 C. B. S. Rao, 'Chemistry of Lignans,' Andhra University Press, 1978.
2 S. R. Stitch, J. K. Toumba, M. B. Groen, C. W. Funke, J. Leemhuis, J. Vink, and G. F. Woods, *Nature (London)*, 1980, **287**, 738.
3 K. D. R. Setchell, A. M. Lawson, F. L. Mitchell, H. Adlercreutz, D. N. Kirk, and M. Axelson, *Nature (London)* 1980, **287**, 740.
4 K. D. R. Setchell, A. M. Lawson, E. Conway, N. F. Taylor, D. N. Kirk, G. Cooley, R. D. Farrant, S. Wynn, and M. Axelson, *Biochem. J.*, 1980, **197**, 447.
5 M. Axelson and K. D. R. Setchell, *FEBS Lett.*, 1981, **123**, 337.
6 L. Dehennin, A. Reiffsteck, M. Jondet, and M. Thibier, *J. Reprod. Fertil.*, 1982, **66**, 305.
7 K. D. R. Setchell, A. M. Lawson, S. P. Borriello, R. Harkness, H. Gordon, D. L. M. Morgan, D. N. Kirk, H. Adlercreutz, L. C. Andersson, and M. Axelson, *Lancet*, 1981, (ii), 4.
8 K. D. R. Setchell, R. Bull, and H. Adlercreutz, *J. Steroid Biochem.*, 1980, **12**, 375.
9 K. D. R. Setchell, A. M. Lawson, M. Axelson, and H. Adlercreutz, *Res. Steroids*, 1980, **9**, 207.
10 S. R. Stitch, P. D. Illingworth, and J. K. Toumba, *Endocrinology*, 1980, **85**, 23P.
11 M. Axelson, K. D. R. Setchell, *FEBS Lett.*, 1980, **122**, 49.
12 M. Axelson, J. Sjovall, B. E. Gustaffson, and K. D. R. Setchell, *Nature (London)*, 1982, **298**, 659.
13 M. B. Groen and J. Leemhuis, *Tetrahedron Lett.*, 1980, **21**, 5043.
14 K. D. R. Setchell, A. M. Lawson, L. M. McLaughlin, S. K. Patel, D. N. Kirk, and M. Axelson, *Biomed. Mass Spectrom*, 1983, **10**, 227.
15 G. Cooley, R. D. Farrant, D. N. Kirk, and S. Wynn, *Tetrahedron Lett.*, 1981, **21**, 349.
16 A. Pelter, P. Satyanarayana, and R. S. Ward, *Tetrahedron Lett.*, 1981, **22**, 1549.
17 P. A. Ganeshpure and R. Stevenson, *Chem. Ind. (London)*, 1981, 778.
18 K. K. Mahalanabis, M. Mumtaz, and V. Snieckus, *Tetrahedron Lett.*, 1982, **23**, 3975.
19 A. Pelter, P. Satyanarayana, R. S. Ward, and P. Collins, *J. Chem. Soc., Perkin Trans 1*, 1983, 643.
20 F. E. Ziegler and J. A. Schwartz, *J. Org. Chem.*, 1978, **43**, 985.
21 S. Takano and K. Ogasawara, *Synthesis*, 1974, 42.
22 M. Golfier and T. Prange, *Bull. Soc. Chim. Fr.*, 1974, 1158.

* Numbering scheme as for compound (**1**).

Received 22*nd November* 1983; *Paper* 3/2073