SYNTHESIS OF LABELLED NORDIHYDROGUAIARETIC ACID

Carrie Y.-C. Chan, M. A. Leaffer, R. M. Parkhurst and D. W. Thomas

SRI International, Menlo Park, California 94025

L. M. Allen and R. T. Jordan

Chemex Pharmaceuticals, Denver, Colorado 80265

R. S. Pardini

Department of Biochemistry

University of Nevada, Reno, Nevada 89557

SUMMARY

meso-2,3-Dimethyl-1,4-bis(3,4-dihydroxyphenyl)butane-1-^{14}C, (meso-nordihydroguaiaretic acid-1-^{14}C, NDGA-1-^{14}C), was prepared. meso-2,3-Dimethyl-1,4-bis(3,4-dihydroxy-2,5,6-trideuterophenyl)butane (NDGA-d_6, ring labelled) was made from acid exchange of unlabelled natural product and meso-2,3-dimethyl-1,4-bis(3,4-dihydroxyphenyl)-1,1,2,3,4,4-hexadeuterobutane (NDGA-d_6, chain labelled) was prepared from hydrogenation of 3,4-dimethyl-2,5-bis-(3,4-dimethoxyphenyl)furan using deuterium and Pd/C catalyst.

Key Words: Nordihydroguaiaretic-1-^{14}C acid, NDGA-1-^{14}C, 2,3-Dimethyl-1,4-bis-(3,4-dihydroxyphenyl)butane-1-^{14}C, NDGA deuterium labelled, Larrea divaricata, antioxidant.

INTRODUCTION

The discovery that small amounts of meso-nordihydroguaiaretic acid (NDGA) 1 when added to fats and oils afforded excellent antioxidant protection prompted considerable interest in the mid 1940's (1). Early synthetic

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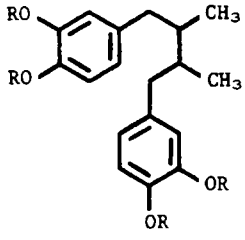
material failed to compete with that extracted from Larrea divaricata, creosote bush, until development of a novel synthetic approach at Hoffmann-LaRoche (2).

NDGA was used as an antioxidant in edible fats for human consumption for thirty years and was considered to be relatively non-toxic; U.S. Food and Drug Administration gave the oral LD_{50} (mice) as 4 g/kg (3). It was finally removed from the GRAS (generally recognized as safe) list as a result of studies that indicated rats receiving a dose of 1-3% in the diet could develop kidney cysts (4,5). This action has now limited the commercial use of NDGA to cosmetic and food products outside the United States and Canada.

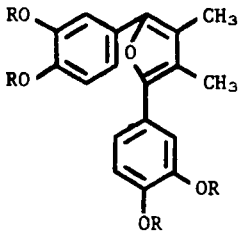
A wide range of interesting biological activity attributable to NDGA has been discovered by many workers. NDGA is a potent inhibitor of lipoxygenase and has shown activity against catechol O-methyltransferase, phenylalanine hydroxylase, succinoxidase among other biological properties. Oliveto has reviewed the chemistry and biology of NDGA (6) and a book on the various chemical components in the creosote bush appeared in 1977 (7). Only recently has the distribution and metabolism of NDGA been studied (8) and has a method been developed for the detection of NDGA in tissue at the picogram level (9) based on methodology developed for the assay of vitamin E (10). With these interests in mind we have prepared the ^{14}C labelled and deuterium labelled nordihydroguaiaretic acid.

DISCUSSION

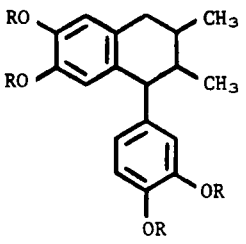
The synthesis of labelled NDGA follows the procedure already known to produce the meso product by a highly stereoselective alkylation (2) and starts with the condensation of propionyl-1-^{14}C chloride with veratrole. The condensation of the 1-(3,4-dimethoxyphenyl)-1-propanone-1-^{14}C formed with unlabelled α-bromo-3,4-dimethoxypropiophenone yields the dl-2,3-dimethyl-1,4-bis(3,4-dimethoxyphenyl)-1,4-butandione-1-^{14}C in good yield and treatment of the diketone with acid gives 3,4-dimethyl-2,5-bis(3,4-dimethoxyphenyl)furan-2-^{14}C 2 also in high yield.



1 R = H
3 R = CH_3



2 R = CH_3



4 R = CH_3
5 R = H

Room temperature hydrogenation of the furan 2 at 750 psi using a large excess of Pd/C catalyst in dry THF gave the meso-NDGA tetramethyl ether 3 in 65% yield. A large excess of catalyst seems to be required and higher temperatures led to reduction of the aromatic ring. One of the major by-products has been reported (2) to be dl-isogalbulin 4 that can be difficult to remove if formed in amounts sufficient to prevent crystallization. The treatment of meso-NDGA tetramethyl ether with 48% HBr produces the demethylated product in almost quantitative yield. The ^{1}H and ^{13}C NMR spectra of product prepared by this method have been shown to be identical with the natural meso form. The meso and dl-NDGA can be easily distinguished since the racemic form melts 30°C lower and shows characteristic differences in the IR spectrum as well as the expected shifts in the chain protons in the ^{1}H NMR and upfield steric compression shifts of 2.3 ppm in the methyl carbons and appropriate downfield shifts in other chain carbons in the ^{13}C NMR. Synthesis of dl-NDGA and derivatives will be reported elsewhere.

Synthesis of meso-2,3-dimethyl-1,4-bis(3,4-dihydroxyphenyl)-1,1,2,3,4,4-hexadeuterobutane by deuteration of the 3,4-dimethyl-2,5-bis(3,4-dimethoxy phenyl)furan 2 using a procedure similar to that used for the synthesis of the ^{14}C-labelled material gave only a 27% yield of the expected NDGA tetramethyl ether. A 10% yield of labelled dl-isogalbulin in the product proved to be

difficult to remove. Demethylation of this mixture with 48% HBr gave meso-2,3-dimethyl-1,4-bis(3,4-dihydroxyphenyl)-1,1,2,3,4,4-hexadeuterobutane that could be easily separated chromatographically from its by-product 5. As expected the distribution of deuterium did not change during the treatment with 48% HBr. The treatment of meso-NDGA with concentrated deutero mineral acids exchanged the ring protons without evidence of exchange of the chain protons.

EXPERIMENTAL

Materials and Methods - Melting points were obtained on a Fisher-Johns melting point block and are uncorrected. ^{13}C NMR spectra were determined on JEOL FX90Q Fourier transform spectrometer using $CDCl_3$ or $CDCl_3$-CD_3OD 1:1 solutions of the labelled compounds. ^{1}H NMR were run on either the JEOL or a Varian EM390 spectrometer. Product purity and reaction progress were detected with analytical thin-layer chromatograph (5 × 20 cm × 250 μ) Analtech plates coated with silica gel GF. Mass spectra were determined using a Ribermag R10-10C gas chromatograph-mass spectrometer system equipped with a 15 meter capillary coated with DB-5 (J & W, Inc.) operating isothermally at 270°C. Infrared spectra were taken using a Perkin-Elmer 137 NaCl spectrophotmeter. Scintillation counting was done with the Beckman LS-250 liquid scintillation system.

1-(3,4-Dimethoxyphenyl)-1-propanone-1-^{14}C. A 50-ml three neck flask equipped with a magnetic stirrer was flushed with argon. To this argon filled flask was added anhydrous aluminum chloride (960 mg, 7.2 mmol), followed by an injection of 6 ml of alcohol free chloroform through a septum. The flask containing the mixture was evacuated while it was kept frozen under liquid nitrogen. Propionyl-1-^{14}C chloride (387 mg, 4.18 mmol, 84 mCi, Wizard Laboratories) was distilled into this frozen mixture. The mixture was then thawed to 0-5° and stirred for 15 min in an ice bath. Argon was bled into the system again to bring it back to atmospheric pressure and a solution of

veratrole (585 mg, 4.23 mmol) in 1 ml of chloroform was injected. The reaction mixture was allowed to stir for 1 hr in an ice bath, decomposed with 3N HCl (7 ml) and extracted with chloroform (10 ml). The chloroform extract was washed with 3N NaOH (5 ml). The two aqueous solutions were back-extracted in succession with $CHCl_3$ (2 × 15 ml). The combined $CHCl_3$ extract was dried over anhydrous $MgSO_4$, and evaporated. The residue which was crystallized from EtOAc:hexane (1:6) (4 ml) gave 505 mg of 1-(3,4-dimethoxyphenyl)-1-propanone-1-^{14}C in 2 crops (2.6 mmol, 62% from propionyl chloride)as colorless crystals, mp 58-60°, Lit. mp 58.5-59.5° (2).

dl-2,3-Dimethyl-1,4-bis(3,4-dimethoxyphenyl)-1,4-butandione-1-^{14}C. To 10 mg powdered $FeCl_3$, was added liquid ammonia (10 ml) followed by a small piece of sodium metal (700 mg, 29.2 mmol). The mixture was allowed to stir for 10 min before solid 1-(3,4-dimethoxyphenyl)-1-propanone-1-^{14}C- (505 mg, 2.6 mmol) was added. The mixture was stirred for another 10 min and solid α-bromo-3,4-dimethoxypropiophenone (0.71 g, 2.6 mmol) was added. After the reaction mixture was stirred for 1 hr, solid ammonium chloride (400 mg) was added, followed by dichloromethane (10 ml), and the grey mixture was allowed to warm to room temperature to evaporate most of the ammonia. The mixture was filtered and the solid residue was extracted with CH_2Cl_2 and the combined filtered extract was evaporated *in vacuo* to an oil which was recrystallized from methanol to give 850 mg of dl-2,3-dimethyl-1,4-bis(3,4-dimethoxyphenyl)-1,4-butandione-1-^{14}C (2.2 mmol, 85% yield) as colorless crystals, mp 137-141°, Lit. mp 145-146° (2).

3,4-Dimethyl-2,5-bis(3,4-dimethoxyphenyl)furan-1-^{14}C 2. The dl-2,3-dimethyl-1,4-bis(3,4-dimethoxyphenyl)-1,4-butandione-1-^{14}C (850 mg, 2.2 mmol) was taken up in 2.7 N ethanolic hydrochloric acid (20 ml) and was allowed to reflux for 40 min. The reaction mixture was allowed to cool to room temperature. The precipitate obtained was filtered, washed with absolute

ethanol and dried in vacuo to give furan 2 (790 mg, 2.13 mmole, 96% yield) as colorless crystals, mp 169-171°, Lit. mp 169-170.5° (2).

meso-2,3-Dimethyl-1,4-bis(3,4-dimethoxyphenyl)-butane-1-^{14}C 3. The furan 2 (790 mg, 2.13 mmol) was added to 10 ml of anhydrous THF and 676 mg of 10% Pd/C (Koch-Light) was added to the mixture. This mixture was stirred in a glass lined Parr bomb overnight at room temperature under 750 psi hydrogen pressure. The catalyst was filtered and washed with THF. The combined filtered solution was evaporated in vacuo to an oil which was recrystallized from methanol to give 494 mg (1.38 mmol, 65% yield) of 3 as colorless crystals, mp 101-104°, Lit. mp 93-95° (2).

meso-2,3-Dimethyl-1,4-bis(3,4-dihydroxyphenyl)butane-1-^{14}C 1. Concentrated hydrobromic acid (4 ml) was added to 494 mg of NDGA tetramethyl ether 3 and the mixture was heated at 130-135° for 9 hr with magnetic stirring and under vacuum in a sealled carius tube. After cooling to room temperature, the tube was opened and the white precipitate was collected by filtration and washed with water until no more bromide ion was detected. After drying under vacuum, meso-NDGA (0.415 g, 1.37 mmole, 98% yield, sp. act. 20.2 mCi/mmol; total act. 27.7 mCi) mp = 186-189°, Lit. mp 184-186° (2) was obtained as colorless crystals and TLC and radioautogram showed it was at least 98% pure (silica gel F-254 on plastic sheet; hexane:acetone 1:1).

meso-2,3-Dimethyl-1,4-bis(3,4-dihydroxy-2,5,6-trideuterophenyl)butane 1. To 20 ml of D_2O refluxing under nitrogen was added dropwise 10 ml of phosphorus oxychloride. To this mixture was added NDGA, 400 mg, and refluxing was continued for 7 hr. The mass spectrum of a small sample of the insoluble product indicated that about 12% had exchanged completely (NDGA-d_6) and 88% of this product was NDGA-d_0. Ten milliliters of CH_3OD was added to the mixture to aid the solubility of the NDGA and refluxing continued for 3 hr more. The

methanol was evaporated from the mixture with a slow stream of nitrogen and the cooled solution was filtered; washed with D_2O to give 402.9 mg of colorless product, mp = 186-187°C, R_f = 0.65-0.71 acetone:hexane 1:1. Mass spectral analysis of the product as its tetrakis(trimethylsilyl) derivative indicated that 92% was completely ring labelled (NDGA-d_6) and 8% was NDGA-d_5 with no lesser deuterated NDGA. MS (%) 601 (2), 600 (6), 599 (20), 598 (40), 597 (80), 596 (100), 595 (10), 584 (2), 583 (5), 582 (10), 581 (12), 274 (1), 273 (2), 272 (10), 271 (20) 270 (45), 269 (12), 184 (2), 183 (10), 182 (32), 181 (5). ^{1}H-NMR ($CDCl_3$:CD_3OD, 1:1-TMS) δ 0.78 (6H, d, J = 6 Hz), 1.66 (2H, poorly resolved q, J = 6 Hz), 2.12 (2H, unsymmetrical q, J_1 = 13, J_2 = 9 Hz), 2.61 (2H, unsymmetrical q, J_1 = 12, J_2 = 6 Hz). IR (mull) 3.89, 4.04, 4.17, 6.28, 7.15, 7.25, 7.92, 7.98, 8.34, 8.46, 9.37, 9.78, and 10.84 μ. ^{13}C-NMR ($CDCl_3$-CD_3OD_2 ref. 77.000) 15.30, 37.83, 38.65, 133.23, 142.90, 144.80.

meso-2,3-Dimethyl-1,4-bis(3,4-dihydroxyphenyl)1,1,2,3,4,4-hexadeuterobutane 1. To 400 mg of 3,4-dimethyl-2,5-bis(3,4-dimethoxyphenyl)furan (6) and 400 mg of 10% Pd/C (Koch-Light) in 5 ml of THF (dried by distillation from LAH) was added a magnetic stirrer and the glass vial was placed in Parr bomb and pressurized at 1000 psi with deuterium gas. This mixture was stirred for several days at room temperature. TLC of the reaction mixture indicated only a small conversion. The bomb was repressurized and the reaction continued for a total of 4 weeks. The catalyst was filtered and the solvent evaporated in a slow stream of dry nitrogen. GC-MS showed a complex mixture of products where the expected chain labelled product represented only 27.6% of the total (m/e M^+ 364). Other products M^+ = 367 (2.9), 366 (9), 365 (29), 364 (100), 363 (32), 362 (12), 361 (10.6%). Two isomers of M^+ = 376 (9.7% and 15.3%), four isomer of M^+ = 347 (5.7%, 11.8%, 2.7% and 3.1%) and M^+ = 318 (6.4%). The product was isolated by thin layer chromatography on silica-gel first with chloroform as a solvent (R_f = 0.35). The band was eluted with acetone and applied to a second silica-gel plate. The second plate was chromatographed

with ether-hexane (1:1) as the solvent (R_f = 0.29) and the band was eluted with acetone. Evaporation of the solvent gave 136.7 mg of colorless oil. GC-MS of this purified product showed that it still was contaminated with the dl-deutero-isogalbulin (M^+ = 361). Mass spectral analysis of the meso-NDGA tetramethyl ether 3 indicated 67% NDGA-d_6, 21% NDGA-d_5 and 7% NDGA-d_4. The crude chain labelled meso-NDGA tetramethyl ether 3 obtained above (130 mg) from which the solvent had been carefully removed in a vacuum and 2 ml of 48% HBr that had been freshly boiled to remove bromine were frozen in liquid nitrogen and sealed in a vacuum tube of about 10 ml capacity. The contents of the tube were stirred magnetically in a bath at 130-135° for 9 hr. The tube was removed, opened and 20 ml of distilled water added. The black tarry residue and diluted hydrobromic acid was extracted twice with ether, the ether washed with saturated sodium bicarbonate and dried over sodium sulfate. The ether solution was evaporated to a small volume and placed on four 20 × 20 cm × 250μ silica gel plates and run in acetone:hexane (1:1). The lowest UV absorbing band (R_f 0.6) was eluted with acetone and the residue recrystallized from methanol:water 1:3 to give 39 mg; 36% yield of colorless plates, m.p. = 185-186°C. Mass spectral analysis of the product as its TMS ether derivative indicated 65% NDGA-d_6, 22% NDGA-d_5 and 7% NDGA-d_4. MS (%) 601 (1), 600 (5), 599 (15), 598 (40), 597 (62), 596 (100), 595 (31), 594 (10), 584 (2), 583 (6), 582 (10), 581 (14), 580 (6), 579 (1), 298 (4), 273 (2), 272 (10), 271 (20), 270 (40), 269 (7), 268 (20), 267 (4), 183 (4), 182 (12), 181 (40), 180 (6), 179 (1). Major impurity at 1.7% M^+ = 610. ^{1}H-NMR ($CDCl_3$-CD_3OD, ref. TMS) 0.83, δ; 6.42-6.90 mult. ppm. ^{13}C-NMR ($CDCl_3$-CD_3OD, ref. 49.000) 16.23, 115.58, 116.61, 121.11, 134.43, 142.99 and 144.89.

REFERENCES

1. Lundberg, W. O., Halvorson, H. O., and Burr, G. O. Oil and Soap 21, 33 (1944).
2. Perry, C. W., Kalnins, M. V., and Deitcher, K. H. J. Org. Chem. 37, 4371 (1972).

3. Lehman, A. J., Fitzhugh, O. G., Nelson, A. A., and Woodward, G. In Advances in food research, Vol. III, E. M. Mrak and G. F. Stewart (eds.), 1951, Academic Press, New York, p. 197-208

4. Goodman, T. Grice, H. C., Becking, G. C., and Salem, F. A. Lab. Invest. 23, 93 (1970).

5. Evan, A. P. and Gardner, Jr., K. D. Kidney Int. 15, 7 (1979).

6. Oliveto, E. P. Chemistry and Industry, September 2, 1972, p. 677.

7. Creosote Bush-Biology and Chemistry of Larrea in New World Deserts. Ed. T. J. Mabry, J. H. Hunziker, and D. R. DiFeo, Jr., Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pennsylvania, 1977. Distributed by Academic Press.

8. Personal communication. Dr. J. H. Peters, SRI International. Allen, L. M., Parkhurst, R. M., Leaffer, M.A., Steeger, T. M., and Peters, J. H., to be presented UPHAR 9th International Congress of Pharmacology.

9. Personal communication, Dr. R. J. Stephens, SRI International.

10. Thomas, D. W., Parkhurst, R. M., Negi, D. S., Lunan, K. D., Wen, A. C., Brandt, A. E., and Stephens, R. J. J. Chromatog. (Biomed. Appl.) 225, 433-439 (1981).