Synthesis of Deuterated Grandinol

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Received May 14, 1984

Grandinol (1) was initially found as a rooting inhibitor in adult tissues of Eucalyptus grandis, but has subsequently been detected as a common active principle in various Eucalyptus species in our search for germination inhibitors within the genus. The grandinol content seemed to differ with the type (sub-species) of Eucalyptus and/or the tissue parts, so it seemed possible that information of some interest for plant-physiology and/or chemotaxonomy might emerge from a quantitative survey for grandinol. In order to carry out this study, a suitable internal standard such as an isotope-labelled compound is required for reliable results. In this communication, we report the synthesis of deuterated grandinol (2) using a new route.

The total synthesis of 1 from trinitrotoluene can be achieved in only three steps; however this synthetic route is not suitable for introduction of the methyl instead of the aryl methyl of 1. Phlorisovalerophenone (3) was initially selected as a starting material, but many attempts at nuclear methylation of 3 were unsuccessful, due to the very high reactivity of the two vacant positions. In an alternative formylation approach of 3, using ethyl orthoformate and aluminum chloride in methylene chloride, 3-formylphlorisovalerophenone (4) was given as a major product, which has only one aromatic proton to be replaced by the methyl group at the last step. Treatment of 4 with excess methyl iodide and potassium hydroxide in 10% aqueous methanol achieved a methyl substitution to give 1. Having established the new route to 1, 2 was synthesized in a similar manner, using d3-methyl iodide in 10% D2O in CD3OD for the methylation of 4.

In GC/MS analysis, 1 and the deuterated grandinol were converted to trimethylsilyl (TMS) ethers by a treatment with bistrimethylsilylacetamide (BSA). TMS ethers of 1 and the deuterated grandinol gave rather simple spectra in both the electron-impact and chemical ionization methods (EI and CI respectively). As the fragments M−15 were base peaks in the EI mass spectra, the protonated molecular ions were dominant in the CI mass spectra (Fig. 1). The CI mass chromatogram monitored at m/z 469 to 475 clearly revealed the incorporation ratio of deuterium in the deuterated grandinol (d1 : d2 : d3 = ca. 2: 2: 1, a peak was not observed for d0). Some additional deuteration on the ketonic side chain of 2 could be expected under the reaction conditions, which would be more desirable for our purpose. For wide range quantitation, the internal standard for GC/MS measurement must have distinctive mass differences as large as possible to avoid the effects of 13C isotopes in the natural sample (in the case of the trimethylsilyl ether of grandinol, the intensity ratios of M+ + 1, M+ + 2, M+ + 3 and M+ + 4 to M+ are 24.4, 5.7, 1.3 and 0.3% respectively). The labelled pattern of the deuterated grandinol was not changed throughout the purification procedure using silica gel Sep-Pak and HPLC, which have been adopted for the natural samples. We have now attained the internal standard for quantitative analysis of 1 in Eucalyptus extracts, and the work is in progress.

EXPERIMENTAL

GC/EIMS and GC/CIMS were carried out with a Jeol DX-300 mass spectrometer under the following conditions. GC: column, OV-1, 1 or 2 m; oven temperature, 220°C for 1 m and 230°C for 2 m columns; flow rate, 20 ml/min for El and 10 ml for CI. CIMS: reactant gas, isobutane; chamber pressure, 1.33 x 10−3 Pa=1 x 10−5 Torr; scan speed, 1 sec for m/z 50 to 600; chamber temperature, ambient.

i) 3-Formylphlorisovalerophenone (4). In a 100 ml flask, 7.5 g (55 mmol) of anhydrous aluminum chloride was mixed with a solution of 1.05 g (50 mmol) of phlorisorovalerophenone (dried over phosphorus pentoxide in vacuo) in methylene chloride (50 ml), and the mixture was stirred for 10 min at room temperature. The flask was cooled in an ice bath and a solution of ethyl orthoformate (7.5 g, 50 mmol) in 25 ml of methylene chloride was added.

Scheme 1. Synthetic Route for Grandinols.
The reaction mixture was stirred for 30 min and then poured onto crashed ice (100 g). The product was extracted with methylene chloride (200 ml x 5), and the organic layer was dried with anhydrous sodium sulfate and evaporated to dryness. The solid residue was purified by silica gel chromatography using 20% ethyl acetate in hexane as a solvent, and pure 4 was obtained in a 70% yield. mp 74°C; νmax (Nujol) cm⁻¹: 3350, 1650, 1630, 1600; 1H NMR (CDCl₃) δ ppm: 0.96 (6H, d, J = 7 Hz), 2.22 (1H, m, J = 7 and 8), 2.97 (2H, d, J = 8), 5.84 (1H, s), 10.04 (1H, s); MS m/z: 238 (M⁺, 28.5%), 223 (18.6), 181 (100).

Deuterated grandinol. In a 4 ml vial which could be tightly sealed with a screw cap, a mixture of 4 (238 mg, 1 mmol), potassium hydroxide (230 mg, approx 4 mmol), D₃-iodomethane (0.5 ml, approx 8 mmol), d₄-methanol (2 ml) and deuterium oxide (0.5 ml) was placed to be heated at 70°C for 4 hr. After methylation, 15 ml of 5% aqueous methanol was added to the mixture, which was then extracted with hexane (10 ml x 5). The hexane extracts were combined, concentrated and then applied onto a silica gel column (1.5 cm i.d. x 10 cm). Deuterated grandinol was eluted with 10% ethyl acetate in hexane, and recrystallized from hot hexane to obtain 72 mg of colorless needles; yield approx 28%; mp 125-130°C; λmax (EtOH): 278 nm (ε 28200), 345 (ε 4100); νmax (Nujol): 3340, 1650, 1630 and 1600 cm⁻¹; δ(CDCl₃): 1.00 (6H, d, J = 7 Hz), 2.25 (1H, m), 10.3 (1H, s); MS m/z: 257 (d₅-M⁺, 15%), 256 (d₄-M⁺, 27), 255 (d₃-M⁺, 20), 198 (100).

Acknowledgments. This study was funded by the Science and Technology Agency (JAPAN) and Department of Science and Technology (AUSTRALIA) as a "Co-operative Research Project on Plant Growth Regulators in Tropical and Subtropical Plants." We wish to express our thanks to Institute of Physical and
Chemical Research for their support of this research.

REFERENCES


