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Isolation of s-Butyl β-D-Glucopyranoside from *Acripeza reticulata*

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Abstract

s-Butyl β-D-glucopyranoside has been identified as a major component in the aqueous secretion collected from the dorsal surface of the abdomen of the tettigoniid, *Acripeza reticulata* Guér. The compound has a bitter taste and may fulfil a defensive role in the insect.

The tettigoniid, *Acripeza reticulata* Guér. (Orthoptera), occurs widely in the highland areas of the Australian Capital Territory and Snowy Mountains. The female is incapable of flight, having only small shell-like forewings, while the male has fully developed wings. When the insect is disturbed the wings 'can be raised to reveal bright coloured bands on the abdomen which presumably serve an aposematic function'.[1]

Behavioural studies with *Gymnorhina tibicen* (the black-backed magpie) have shown that if the bird seizes an *A. reticulata* despite the display, the insect is quickly discarded and the attacker engages in vigorous wiping of the beak on the ground or a branch of a tree. The bird will not approach a second *A. reticulata* for a period of some days following the initial contact.[2] We were interested in the chemical basis of this repulsion since the insect does not appear to discharge any highly odoriferous or corrosive secretion in response to attack, as is common in a number of other orders of insects.[3]

Close examination of the insects revealed a number of small droplets of a viscous liquid possessing a faint odour and a bitter taste on the dark areas along the dorsal surface of the highly coloured abdomen. These aqueous droplets were collected by means of a fine glass capillary. We were unable to detect any volatile components by gas chromatography of the collected material, despite the faint odour. Thin-layer chromatography on silica gel coated plates developed with 20% methanol in chloroform indicated one major and several minor components. The compounds appeared as bluish purple spots on the t.l.c. plates, when sprayed with anisaldehyde–sulphuric acid reagent.[4]

The principal component was obtained in a pure state by column chromatography on silica gel but proved thermally unstable under the conditions encountered in the

[1] Key, K. H. L., 'Insects of Australia' p. 336 (Melbourne University Press 1970).
[2] Nocke, H., unpublished data.
[3] Eisner, T., 'Chemical Ecology' p. 157 (Eds E. Sondheimer and J. B. Simeon) (Academic Press: New York 1970).
[4] Stahl, E., 'Thin-Layer Chromatography' p. 485 (Academic Press: New York 1965).

mass spectrometer. The p.m.r. spectrum indicated the compound might be a β-alkyl glucoside with the doublet at δ 4·53, *J* 7·5, being assigned as the glycosidic anomeric proton. The integration and the multiplicity of the signals at δ 0·9 (triplet, *J* 7·5, 3H), 1·16 (doublet, *J* 6·5, 3H) and 1·57 (multiplet, 2H) would be satisfied if a s-butyl moiety was assigned as the β-alkyl group.

The crystalline tetraacetate (m.p. 125–126°) obtained on treatment of the glucoside with acetic anhydride and pyridine was stable to the conditions of the gas chromatograph and the mass spectrometer. The mass spectrum of a gas chromatographically pure sample (5% QF-1, 5 ft by ¼ in. stainless steel column at 200°C) was very similar to the published mass spectrum of β-D-glucopyranose pentaacetate.[5]

The elementary compositions of the ions of *m/e* 331, 242 and 200 were determined by high-resolution mass matching to be $C_{14}H_{19}O_9$, $C_{11}H_{14}O_6$ and $C_9H_{12}O_5$ respectively.

To confirm the s-butyl β-D-glucopyranose structure assigned to the component isolated from *A. reticulata*, it was necessary to synthesize this compound for comparison. The reaction of (+)-propylene oxide with lithium dimethyl copper[6] gave (+)-butan-2-ol[7] which was used to prepare (+)-s-butyl β-D-glucopyranoside tetraacetate on reaction with acetobromoglucose. The (−)-diastereomer was obtained from commercially available (−)-butan-2-ol and acetobromoglucose.

The p.m.r. spectrum determined following acetylation of the glycoside obtained from *A. reticulata* indicated that the product was predominantly (−)-s-butyl β-D-glucopyranoside tetraacetate. Preparative gas chromatography of the glycoside acetates gave a pure sample of the major component, the p.m.r. spectrum of which was identical with the spectrum obtained from synthetic (−)-s-butyl β-D-glucopyranoside tetraacetate. The two compounds had identical g.l.c. retention times and melting points (126–127°).

Although the synthesis of both diastereomers of s-butyl β-D-glucopyranoside has been reported in the literature[8] the occurrence of this compound as a natural product has not been reported. Every effort has been made in this work to rule out the possibility of the compound arising as an artefact of the isolation procedure. The occurrence of the compound in the insect secretion as almost exclusively the (−)-isomer tends to reinforce the idea that the compound is a natural product.

Early reports regarding the synthesis of (−)-s-butyl β-D-glucopyranoside noted that the compound possesses a bitter taste so it is likely that it contributes to the taste of the secretion from *A. reticulata*. In our experience the synthetic glycoside has a taste similar to, but not identical with, that of the secretion from *A. reticulata* indicating that there are other compounds as yet unidentified contributing to the bitter taste of this secretion. The glycoside is however the major organic component and may play an important part in defending the insect from aggressors.

Experimental

Melting points were determined on a Kofler hot-stage and are uncorrected. P.m.r. spectra were recorded at 100 MHz on a Varian Associates HA-100 instrument with deuterochloroform solutions.

[5] Budzikiewicz, H., Djerassi, C., and Williams, D. H., 'Structure Elucidation of Natural Products' Vol. 2, p. 205 (Holden–Day: San Francisco 1964).
[6] Herr, R. W., Weiland, D. M., and Johnson, C. R., *J. Amer. Chem. Soc.*, 1970, **92**, 3813.
[7] Golding, B. T., Hall, D. R., and Sakrikar, S., *J. Chem. Soc., Perkin Trans. 1*, 1973, 1214.
[8] Bourquelot, E. M., and Bridel, M., *C. R. Acad. Sci.*, 1913, **155**, 437.

The chemical shifts were measured on the δ scale relative to tetramethylsilane as the internal standard. Mass spectra were determined on an AEI MS-902 spectrometer at 70 eV with the samples being introduced by the direct probe. Optical rotations in ethanol were measured on a Bellingham and Stanley polarimeter with a 1-dm cell.

Acetylation of the Glucoside from A. reticulata

The aqueous solution collected from the dorsal surface of the insect was evaporated to dryness at room temperature. The residue was dissolved in acetic anhydride/pyridine (1 : 1), maintained at 5°C for a period of 14 h and then poured over crushed ice. The aqueous mixture was extracted with ether (3 ×) and the combined ether extracts washed with dilute HCl and water prior to drying with anhydrous sodium sulphate. The solid remaining after evaporation of the ether was crystallized from ether–hexane giving needles, m.p. 126–127°.

Synthesis of (−)-s-Butyl β-D-Glucopyranose Tetraacetate

This compound was synthesized from acetobromoglucose and (−)-butan-2-ol following the procedure of Lindberg.[9] The product was obtained crystalline from ether–hexane as colourless needles, m.p. 126–127°, $[\alpha]_D$ −37·7° (lit.[10] m.p. 126–127°, $[\alpha]_D$ −36·6°).

Synthesis of (+)-s-Butyl β-D-Glucopyranose Tetraacetate

This compound was prepared from acetobromoglucose and (+)-butan-2-ol[7] as in the procedure above. The product was obtained as colourless needles, m.p. 102°, $[\alpha]_D$ −19·1° (lit.[10] m.p. 101–103°, $[\alpha]_D$ −18·5°).

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[9] Lindberg, B., *Acta Chem. Scand.*, 1949, **3**, 151.
[10] Viebel, S., and Lillelund, H., *Bull. Soc. Chim. Fr.*, 1938, **5**, 494.