

species have been fully identified. Of these, cyanidin 3-glucoside has been confirmed in six species, i.e. *Hordeum vulgare*, *Oryza sativa*, *Pennisetum japonica*, *Poa annua*, *Sorghum vulgare*, and *Zea mays*.²

Cyanidin 3-glucoside has now been identified in leaves of *Oropetium thomaeum* (L.f.) Trin., a dominant component of the ground cover vegetation of Delhi Ridge, and the neighbouring rocky and hilly tracts (Dakshini and Tandon³). This grass is quite peculiar in changing its colour from green to pink-violet when dry, and can withstand the extreme conditions of drought. It is possible that the presence of cyanidin 3-glucoside in *O. thomaeum* and other grasses is of value in their adaptability to drought resistance, either by shielding the chloroplasts from intense sunlight and protecting them from degeneration, or through some other mechanism.

EXPERIMENTAL

Plant source. Leaves of *Oropetium thomaeum* were collected from the Old Delhi Ridge, opposite the University campus, from Delhi, India, and were air dried before extraction.

Flavonoid identification. The flavonoid was isolated and identified, by standard procedures,² by direct comparison with authentic pigment and by identification of the products of acid hydrolysis.

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Key Word Index—*Oropetium thomaeum*; Gramineae; cyanidin-3-glucoside.

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LILIACEAE

γ -METHYLENEGLUTAMIC ACID FROM *LILIUM CANDIDUM* BULBS

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γ -METHYLENEGLUTAMIC ACID was first isolated from *Arachis hypogaea*¹ and later from *Tulipa gesneriana*,^{2,3} *Phyllitis scolopendrium*,⁴ *Amorpha fruticosa*,⁵ *Tetrapleura tetraptera*⁶

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and *Lilium maximowiczii*.⁷ Its presence has also been recognized in *Humulus lupulus*,⁸ *Lilium regale*,⁹ *L. longiflorum*, *Notholirium thompsonianum*, *Fritillaria meleagris*, *Haworthia coarctata*,¹⁰ *Polygala vulgaris*¹¹ and *Tamarindus indica*.¹²

Our previous work on *Lilium candidum* has shown the presence of a compound with chromatographic behaviour identical to that of γ -methyleneglutamic acid, which accumulated in large quantities in the roots and in the bulbs at the time of flowering.^{13,14} In *Lilium candidum* bulbs, we also observed¹⁵ a compound with antimycotic activity and the same chromatographic characteristics as γ -methylenebutyrolactone *. The full identification as γ -methyleneglutamic acid was therefore of interest, in view of its possible metabolic relationship with γ -methylenebutyrolactone and related substances in the bulbs.

The acid was isolated in a pure form by chromatography on Dowex 1 \times 8 columns and repeated crystallization from water. It was identified by detailed comparison with authentic material (see Experimental).

EXPERIMENTAL

Isolation technique. 10 kg of *Lilium candidum* bulbs collected at the time of flowering were macerated in a domestic blender and extracted for 24 hr at 4° with 22 l. 95% EtOH. The extract was filtered and evaporated to about 1 l. at 50°. This extract was applied in 200 ml lots to a Dowex 1 \times 8 column (acetate form, mesh 60–100, 50 \times 7 cm), which was then eluted with 2 l. each of 0.1 N, 0.2 N and 0.4 N HOAc, and 4 l. 0.5 N HOAc. Eluents with 0.4 and 0.5 N HOAc were collected in 20 ml fractions. All these fractions were monitored by TLC on silica gel in *n*-BuOH-HOAc-H₂O (6:2:2). The fractions containing the new acid were combined and evaporated *in vacuo*. This material (about 4 g) was dissolved in water, decolourized with charcoal, and recrystallized four times from water (yield 0.650 g).

Oxidation with KMnO₄. A 40 mg sample was dissolved in 20 ml of 5% H₂SO₄ and the solution was added dropwise at room temp. to 0.01 N KMnO₄ until the appearance of a persistent pink colour, heated at 50° and again treated with KMnO₄. The solution was then filtered and the filtrate was examined for aspartic acid by means of two-dimensional TLC.

Catalytic reduction. An aqueous solution of the isolated material (180 mg in 10 ml H₂O) was treated with hydrogen at 20° in the presence of 25 mg PtO₂; reduction was complete in 30 min. The resulting solution was examined for γ -methylglutamic acid by means of two-dimensional TLC.

Properties of the isolated compound. The isolated compound gave a yellow-brown colour on silica gel plates when sprayed with 0.1% ninhydrin in acetone. Samples of the compound are unaltered by 24 hr hydrolysis with 6 N HCl in sealed tubes. When crystallized from water, it melted at 197° and the elemental composition: C, 40.88; H, 6.28; N, 7.99. (Required for C₆H₂NO₄·H₂O; C, 40.68; H, 6.26; N, 7.91%). After drying it melted at 310–320°, with decomp. and the elemental composition: C, 45.52; H, 5.75; N, 8.59. (Required for C₆H₉NO₄: C, 45.28; H, 5.70; N, 8.80%). It was identical to an authentic sample of γ -methyleneglutamic acid in m.p., chromatographic behaviour and in IR and NMR spectra.

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Key Word Index—*Lilium candidum*; Liliaceae; γ -methyleneglutamic acid.