Phytochemistry, 1971, Vol. 11, pp. 2589 to 2591. Pergamon Press. Printed in England.

SHORT COMMUNICATION

MONOTERPENE GLUCOSIDES FROM PETALS OF *TANACETUM VULGARE*

D. V. BANTHORPE and J. MANN

Christopher Ingold Laboratories, University College, 20 Gordon Street, London WC1

(*Received* 10 *February* 1972)

Key Word Index—*Tanacetum vulgare*; Compositae; petals; monoterpene glucosides; isothujol; neoisothujol; mevalonate incorporation.

Abstract—Petals of *Tanacetum vulgare* contained monoterpenes (*ca.* 0·06%) of which over half were the β-D-glucosides of isothujol, neoisothujol, and, probably, α-terpineol and terpinen-4-ol. At periods of 1·5 and 21 hr after feeding [2-^{14}C]-mevalonate, incorporations into these conjugated terpenols were *ca.* 0·9 and 0·6%: incorporations into the co-occurring free terpenols were 10-fold less.

INTRODUCTION

GLUCOSIDES possessing a cyclopentanoid monoterpenol as the aglycone are well known,[1] but only recently has the natural occurrence of glucosides of monoterpenols with more conventional skeletons been firmly established. Thus, the β-D-glucosides of geraniol, nerol and citronellol were isolated from petals of *Rosa dilecta* cv. Lady Seton, and the first compound was also extracted from petals of *Rosa damascena* cv. versicolor.[2–4] We now report the occurrence of related compounds in *Tanacetum vulgare* L. (fam. Compositae).

RESULTS AND DISCUSSION

The isolation and hydrolysis studies (Experimental procedures *A* and *B*) show that the petals of *T. vulgare* just after the flower head has opened, contain up to 0·06% of monoterpenes, of which over half are bonded as β-glucosides of isothujol, neoisothujol and probably α-terpineol and terpinen-4-ol. Buds and necrotic blooms contained insignificant amounts of either free or bonded monoterpenes and leaves contained negligible amounts of β-glucosides.

The terpene content of the petals was about one third of that in the leaves.[5] Isothujol and neoisothujol were the major monoterpenols in both tissues; but isothujone, the main (> 80%) component of the leaf oil, was almost absent from the petals.

Our observations of rapid and extensive labelling of the terpenoid moieties of the glucosides on feeding the flower heads with mevalonic acid closely parallel those of the previous workers using *Rosa* species. The incorporations in *T. vulgare* were some 10-fold less than the maximum values reported for the *Rosa* species, but are nevertheless 10^2–10^3-fold greater than the incorporations of the same precursor into the leaf monoterpenes of

[1] V. PLOUVIER, *Phytochem.* **10,** 1697 (1971).
[2] M. J. O. FRANCIS and C. ALLCOCK, *Phytochem.* **8,** 1339 (1969).
[3] M. J. O. FRANCIS and M. O'CONNELL, *Phytochem.* **8,** 1705 (1969).
[4] M. J. O. FRANCIS and C. ALLCOCK, *Biochem. J.* **113,** 38P (1969).
[5] D. V. BANTHORPE and A. WIRZ-JUSTICE, *J. Chem. Soc.* **C,** 541 (1969).

T. vulgare[6] or of other species in general.[7] Incorporation of mevalonic acid into the free terpenols of petals of *T. vulgare* is also 10–10^2 fold greater than the latter values, but the incorporation into isothujone (*ca.* 0·001 %) is comparable to that found in leaf tissue. These latter results are consistent with the view[5] that isothujone is formed from the epimeric thujols.

Our results indicate that petals may be used in the investigation of terpene biosynthesis although the experiments with the *Chrysanthemum* species show that monoterpene glucosides may not be of widespread occurrence.

EXPERIMENTAL

Isolation of β-D-glucosides. Petals from fully-opened flower heads of *T. vulgare* grown in Central London from seed provided by the Royal Botanic Gardens, Kew, were harvested in late August and worked up by two procedures. *Procedure A.* The petals (50 g) were ground in liquid N_2 and the resulting powder was extracted with acetate buffer (0·1 M, pH 7, 75 ml) and the product chromatographed on Silica Gel H (Merck; 15 × 1·5 cm o.d.) with EtOAc–MeOH and further processed by described methods[2] to isolate the fraction containing glucosides ($PhNH_2$–phthalate spray; developed at 100°) and these were further separated by TLC on Silica Gel H with EtOH–EtOAc (18:85); *n*-PrOH–EtOAc–H_2O (6:3:1); and $CHCl_3$–AcOH–MeOH (85:2:13). Two products *A* and *B* (5, 2 mg) were isolated which had m.ps 153°d, 162°d and $[\alpha]_D^{20}$ (*c*, 0·1, H_2O; Ericsson–Bendix electronic polarimeter) +115°, +55°; and these were present (in total) in *ca.* 0·014% of the starting material. *A* and *B* (2 mg) were incubated with β-glucosidase (emulsin *ex* almonds, Sigma) or β-glucuronidase (ex *Helix pomatia*, Sigma: this contains a potent β-glucosidase) for 3 hr at 40° and the products were extracted with light petroleum (b.p. 40–60°, 0·5 ml) and analysed[5,8] by GLC. Isothujol and neoisothujol were the sole extractable products from *A* and *B* respectively as characterized by their retention characteristics on four GLC systems,[5] and the former was cut-out and identified by a micro-IR technique.[9] It had $[\alpha]_D^{25}$ +104° (*c*, 0·1, $CHCl_3$). Both products were also characterized by comparison of their MS with those of authentic specimens. The aqueous fraction from the enzymic incubations yielded a product (*C*) that co-chromatographed with D-glucose in several TLC and PC systems, and in particular had R_f 0·45 on Silica Gel HF 254 with *n*-BuOH–AcOH–Et_2O–H_2O (9:6:3:1) as eluant. (*C*) isolated from (*A*) had $[\alpha]_D^{25}$ +53° (*c*, 0·10, H_2O + trace NH_4OH) and was completely destroyed by β-D-glucose oxidase. Colorimetric determinations[10] and GLC analysis showed that (*A*) contained isothujol: glucose in the ratio 0·9 ± 0·2:1, and an elemental analysis gave: Found C, 60·3; H, 8·8. Calc. for $C_{16}H_{28}O_6$; C, 60·8; H, 8·8%. A carbohydrate-containing fraction (< 0·1 mg) from the original separation was enzymically cleaved as above and gave alcohols (in approx. equal quantities) that had the retention times of α-terpineol and terpinen-4-ol in the four GLC systems. No terpenols were liberated when this last fraction or *A* or *B* were incubated with α-glucosidase (ex yeast, Sigma). *Procedure B.* Petals were ground as in procedure *A* and the product was steam-distilled. The distillate was saturated with NaCl and extracted with light petroleum (b.p. 40–60°; 2 × 50 ml) to give fraction 1. The steam-involatile residue was made pH 2 (H_2SO_4) and heated at 80° for 10 hr, after which the mixture was steam distilled to give a fresh distillate which was worked up to give fraction 2. GLC analysis of fraction 1 and isolation and IR characterisation of the major products gave isothujol (9 mg), neoisothujol (2 mg), terpinen-4-ol (0·5 mg), borneol (0·5 mg) and hydrocarbons (*ca.* 3 mg). Fraction 2 contained isothujol (7 mg), neoisothujol (4 mg), α-terpineol (< 1 mg), terpinen-4-ol (< 1 mg), hydrocarbons (sabinene, terpinolene, α-terpinene 3 mg). Only traces of isothujone (< 0·1 mg) could be detected in fraction 1 and none in fraction 2. Several additional minor hydrocarbons occurred in fraction 2 that were absent in fraction 1; these may have been derived from dehydration of alcohols during the hydrolysis procedure.

Synthesis of terpenyl β-D-glucosides. Isothujol and neoisothujol (100 mg) prepared previously[8] were incubated with β-glucosidase (10 mg) and glucose (1·0 g) for 15 days at 37° using a previously outlined[2,4] procedure. Products were formed in each case in 0·1–0·3% yield which co-chromatographed with the glucosides isolated from petals and which were cleaved with β-glucosidase to the expected products. Previous workers[2,4] found similarly low yields in this procedure.

Occurrence of glucosides. Repetition of procedure B, revealed that conjugated monoterpenols were only present in detectable quantities in petals from open flower heads. None occurred in unopened buds, in blown flowers (i.e. petals freely falling), or in leaves. Such products could not be detected (*ca.* < 0·001 %) in

[6] D. V. BANTHORPE, J. MANN and K. W. TURNBULL, *J. Chem. Soc.* **C**, 2689 (1970).
[7] D. V. BANTHORPE, B. V. CHARLWOOD and M. J. O. FRANCIS, *Chem. Rev.* **72**, 115 (1972).
[8] D. V. BANTHORPE and H. ff. S. DAVIES, *J. Chem. Soc.* **B**, 1356 (1968).
[9] W. J. DE KLEIN, *Analyt. Chem.* **41**, 667 (1969).
[10] W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER, *Manometric Methods and Tissue Metabolism*, 1st Edition, p. 161, Burgess, Minneapolis (1949).

freshly-opened flowers of *Chrysanthemum sinese* cv. Japonica; *C. parthenium L.* or several unidentified *C.* cultivars purchased from local nurserymen.

Tracer studies. Flower heads (*ca.* 4·6 g petals) were placed in [2-^{14}C]-mevalonic acid (sodium salt; 10 μCi; 13 mCi mM^{-1}) and ATP (0·2 mg) in H_2O (2·0 ml) and fed[2–4] using forced transpiration.[5] At 1·5 and 21 hr after commencement of tracer-uptake, the petals were pulverised in liquid N_2 and the free and bonded monoterpenols were extracted using procedure *B*, and were purified by GLC taking precautions[5] against radioactive contamination of the columns and effluents. At 1·5 hr; total activity of terpenols in fractions 1 and 2 were 19 216 (±2%), and 209 968 (±2%) dpm and at 21 hr the values were 12 800 (±12%) and 136 500 (±8%) dpm. Isothujone, extracted with carrier from fraction 1 had 120 dpm. These figures correspond to incorporations of *ca.* 0·9 and 0·6% of the applied tracer into conjugated monoterpenols at the two periods, and *ca.* 0·09 and 0·05% into free terpenols. A control experiment showed that the terpene conjugates in the initially-obtained steam-involatile fraction of the work-up were cleaved by incubation with β-glucosidase to give a free terpene fraction containing greater than 95% of the incorporated tracer (radio-GLC) together with glucose that contained no detectable radioactivity (radio-TLC-scanning). Thus, any labelled terpenyl phosphates or pyrophosphates present in the petals must have been mainly cleaved in the steam-distillation procedure, and only very small quantities of other labelled conjugates (such as the terpenyl esters of carboxylic acids similar to those isolated from petals of *Rosa* species: private communication from Dr. P. Dunphy, Unilever Research, Sharnbrook) could have been present. In addition, incubation of the steam-involatile fraction containing terpene conjugates with a homogenate of leaves of *T. vulgare* that contained phosphatases, or attempted saponification with base gave no evidence for the occurrence of the two latter classes of compounds (*ca.* < 2%, if any).

Acknowledgements—We thank the Science Research Council for a studentship to J. M.; Dr. M. J. O. Francis (Oxford) for helpful discussion; and Mr. C. Gatford and Miss A. Edwards for assistance.