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4-*p*-COUMARYLQUINIC ACID IN APPLE FRUITS

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Previous work indicated that the main isomer of *p*-coumarylquinic acid present in immature fruits of the cider apple cultivar Yarlington Mill was the 3-ester [1, 2]. A second isolation, however, using a silica-gel column, instead of the counter-current method [1], for separation of the isomers gave the 3-, 4- and 5-esters in the approximate ratios 1·0:2·2:1·2 respectively, an unexpected result. Since silica gel does not effect trans-esterification [3] it seemed possible that the 4-ester was the only isomer present, especially since trans-esterification is brought about by $NaHCO_3$ even at 50° [4], and the equilibrium ratios of the 3-:4-:5-isomers are 1:1·15:1·5 [3] after heating at pH 7.

A large-scale extraction was therefore carried out, ensuring that contact with solutions of pH 7 was minimal. Solvent separation of phloridzin at pH 6 was avoided, as even at this pH in the presence of phosphate appreciable trans-esterification occurs with 3-cinnamylquinic acid [3]. The components were therefore separated using Sephadex LH-20. Using this method the *p*-coumarylquinic acid present in the young fruit was found to be almost entirely the 4-ester on the basis of m.p., crystalline form, optical rotation, NMR spectrum, lack of reactivity with periodate and formation of the 3- and 5-esters by $NaHCO_3$ treatment. Examination of cider made from mature cider apples of the same cultivar, using Sephadex LH-20 followed by silica-gel showed only the 4-ester; no 3-ester was detected. It is concluded that in both the immature and mature fruit examined the major isomer of *p*-coumarylquinic acid is the 4-ester with no more than trace amounts of other esters.

EXPERIMENTAL

A sample of young fruits of the cider apple variety Yarlington Mill picked mid-July 1972 was extracted [1] and components separated on a silica-gel column in 0·5 N H_2SO_4 followed by fractional elution [5], (the $CHCl_3$ used to pack the column and the 5–25% *t*-BuOH–$CHCl_3$ mixtures (*t*B–C) used for elution were equilibrated with H_2O instead of 0·5 N H_2SO_4). 3-*p*-Coumarylquinic acid was eluted in the first 40 ml of the 10% *t*B–C, the 4-ester in the last 100 ml of the same mixture, and the 5-ester in the 15–25% *t*B–C. In late June 1973 a sample of fruits at the same stage of development as in 1972 was picked from the same trees, extracted 2× hot *t*-BuOH and the extract filtered and evaporated at 40°. After centrifuging at 27000 *g* for 15 min the extract was made just alkaline with solid $NaHCO_3$ and $Pb(OAc)_2$ (6·5%) was added until no more precipitate was formed; this step was completed as quickly as possible. The liquor was then centrifuged at 3000 *g* for 20 min at 0° and the supernatant adjusted to pH 4·0 with DL-malic acid. The extract was concentrated under vac. at 40° to remove most of the residual *t*-BuOH and the remainder was added to a column of Sephadex LH-20. The column was washed with H_2O until the UV spectrum of the eluate showed the presence of *p*-coumarylquinic acid. MeOH–H_2O (1:4) was then used as eluent followed by MeOH–H_2O (3:7) giving *ca* 0·3% yield. The products from fractions near the beginning, middle and end of the elution were re-crystallized from H_2O with m.ps ranging from 183–184° to 190–192°; their R_f values in 2% HOAc were identical (0·65). The 4- and 5-esters have similar m.ps [4] (192–193° and 193–194°) but differ in their R_f values in 2% HOAc (0·65 and 0·71 respectively for trans forms). (Found C, 55·3; H, 5·4. Calc. for $C_{16}H_{18}O_8 \cdot \frac{1}{2}H_2O$ C, 55·3; H, 5·5%.) $[\alpha]_D^{17}$ −76·7° (*c* 1·0 in MeOH). NMR spectrum of the deuterated Na salt in ^2H_2O showed a double doublet at τ 5·08 (the 3- and 5-esters had this at τ 6·14 and τ 6·22 respectively) (cf Haslam *et al.* [4]). Periodate oxidation [6] had little effect; only 0·07 and 0·09 mol HIO_4 per mole were consumed after 1 and 2½ hr respectively while the corresponding values for the 3-ester were 0·97 and 1·08 mol HIO_4/mol. No more than traces of the 3- ester or 5-ester were detected in the mother liquors. These esters were prepared from the 4-ester by $NaHCO_3$ treatment [4] and were eluted from a silica-gel column before and after the 4-ester respectively. This order of elution, 3-, 4- and then 5-ester, is common to the cinnamyl, *p*-coumaryl and caffeyl derivatives of quinic acid [7]. Fractional elution of the acids in ciders made from three other cultivars showed that the main isomer of *p*-coumarylquinic acid in each was the 4-ester and only trace amounts of the 5-ester were detected.

REFERENCES

1. Williams, A. H. (1958) *Chem. Ind.* 1200.
2. Haslam, E., Howarth, R. D. and Makinson, G. K. (1961) *J. Chem. Soc.* 5153.
3. Hanson, K. R. (1965) *Biochemistry* **4**, 2719.
4. Haslam, E., Makinson, G. K., Naumann, M. O. and Cunningham, J. (1964) *J. Chem. Soc.* 2137.
5. Whiting, G. C. (1958) *J. Sci. Food Agr.* **9**, 244.
6. Dyer, J. R. (1956) *Methods of Biochemical Analysis* (Glick, D., ed.) Vol. 3, pp. 111–152. Interscience Publishers, New York.
7. Hanson, K. R. (1965) *Biochemistry* **4**, 2731.