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3-*O*-SINAPOYLQUINIC ACID

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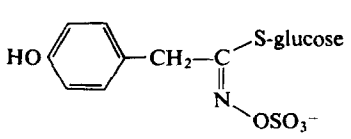
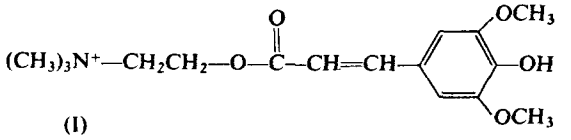
Abstract—3-*O*-Sinapoylquinic acid has been synthesized and its chromatographic and distribution characteristics described.

THE TAXONOMIC significance of sinapic acid has been described by Bate-Smith[1] who found the acid to be widespread in herbaceous plants in an unknown combined form. The only completely characterized conjugate is sinalbin (I) wherein the sinapoyl group is esterified with choline. 1-Sinapoylglucose is reported to be formed on feeding various plants with sinapic acid as well as occurring naturally in *Brassica oleracea*.[2] 1-Sinapoylglucose was not isolated as such, but characterized by its paper chromatographic properties, u.v. spectrum and the formation on hydrolysis of roughly equivalent amounts of sinapic acid and glucose. Similarly, several other sinapic acid esters of glucose have been observed, but not isolated in *Polygala senega* root.[3]





(I)

In 1954 Bate-Smith[4] suggested that ferulic and sinapic acids did not occur esterified with quinic acid, in contrast to caffeic acid. It is not clear what prompted this suggestion. Since then 3-*O*-feruloylquinic acid has been found in coffee beans,[5] tomato stems, leaves and roots,[6] tobacco leaves[7] and sunflower leaves.[8] However, the corresponding 3-*O*-sinapoylquinic acid has not been reported. It seemed possible that the usual survey methods—nearly all dependent on paper chromatography—might not separate 3-*O*-sinapoylquinic acid from other similar and perhaps more abundant compounds. 3-*O*-Sinapoylquinic acid was therefore synthesized, using a modification of the Haslam benzhydryl ester method.[9]

[1] E. C. BATE-SMITH, *J. Linn. Soc.* (*Botany*) **58**, 95 (1962).
[2] J. B. HARBORNE and J. J. CORNER, *Biochem. J.* **81**, 242 (1961).
[3] J. J. CORNER, J. B. HARBORNE, S. G. HUMPHRIES and W. D. OLLIS, *Phytochem.* **1**, 73 (1962).
[4] E. C. BATE-SMITH, *Chem. & Ind.* 1457 (1954).
[5] J. CORSE, E. SONDHEIMER and R. LUNDIN, *Tetrahedron* **18**, 1207 (1962).
[6] J. VAN BRAGT, L. M. ROHRBAUGH and S. H. WENDER, *Phytochem.* **4**, 977 (1965).
[7] A. ZANE, W. STECK and S. H. WENDER, *Tobacco Sci.* **9**, 85 (1965).
[8] A. ZANE and S. H. WENDER, *Nature*, **80**, 209 (1966).
[9] E. HASLAM, R. D. HAWORTH and G. K. MAKINSON, *J. Chem. Soc.* 5153 (1961).

The paper chromatographic properties of 3-*O*-sinapoylquinic acid (Table 1) show it would be masked by chlorogenic acid with the two solvents most commonly used, butanol–acetic acid–water (4:1:5) and 2% acetic acid: R_f 0·62 and 0·55, respectively. Chlorogenic acid in these same solvents, $R_f = 0{\cdot}66$ and 0·58.

Countercurrent distribution might be expected to effect separation, if the right conditions were used. The distribution ratios between ethyl acetate and aqueous solutions of both chlorogenic acid and 3-*O*-sinapoylquinic acid are very susceptible to pH and salt concentration. Oddly enough, freshly prepared solutions of chlorogenic acid, with or without added sodium chloride, when partitioned with reagent quality ethyl acetate show distribution ratios, $K^{10} < 1$. On standing, the chlorogenic acid does partition, probably due to slight hydrolysis

TABLE 1. CHROMATOGRAPHIC AND COUNTERCURRENT DISTRIBUTION PROPERTIES OF CHLOROGENIC AND SOME RELATED ACIDS

	R_f[a]		K (ethyl acetate–sodium chloride solutions)		Reference[b]
	A	B	10% NaCl	1% NaCl	
Chlorogenic acid	0·66; 0·60	0·58; 0·70[c]	0·74[d] (see text)		1
5-Caffeoylquinic acid (neochlorogenic)	0·55	0·61; 0·69[c]	0·18	0·22[e]	1·56
4-Caffeoylquinic acid (band "510")	0·63	0·58; 0·70[c]		0·40[f]	1·22
3-Feruloylquinic acid	0·74[g]	0·61	1·5[d]		0·44
3-*O*-*p*-Coumarylquinic acid	0·78[b]	0·70; (0·80)[d]		1·75[e]	0·69; 0·76
3-*O*-Sinapoylquinic acid	0·72	0·55	1·27[d] (see text)		0·50

[a] Solvent A is BuOH–AcOH–H_2O (4:1:5) upper layer; B is 2 per cent acetic acid.
[b] R_{cg} on silicic Acid (*cg* = chlorogenic acid).[11]
[c] Reference 11; 5 per cent acetic acid.
[d] No pH adjustment.
[e] Ethyl acetate–water; E. HASLAM, G. K. MAKINSON, M. O. NAUMANN and J. CUNNINGHAM, *J. Chem. Soc.* 2137 (1964).
[f] 2 per cent sodium chloride.
[g] BuOH–AcOH–H_2O (4:1:2·2).

of the ethyl acetate. The *K* values for chlorogenic acid between ethyl acetate–water, pH 2·30; ethyl acetate–10% sodium chloride, pH 2·15, and ethyl acetate–10% sodium chloride, pH 2·05, are 0·69, 0·77 and 1·12, respectively. The *K* values for 3-*O*-sinapoylquinic acid between ethyl acetate–water, pH 2·65, is 0·86 and for ethyl acetate–10% sodium chloride is 1·60. It is readily apparent that with the solvent pair ethyl acetate–10% sodium chloride of pH ~ 2·5, the two depsides would be adequately separated after 100 transfers in the Craig apparatus.

Analytical partition colums[11] have proven most valuable in distinguishing the several substituted cinnamoyl esters and this technique shows 3-*O*-sinapoylquinic acid to have an $R_{cg} = 0{\cdot}50$.

Extracts of white mustard seed (*Sinapis alba*) showed no chlorogenic acid-type substances on countercurrent distribution (ethyl acetate–10% sodium chloride). However, relatively

[10] The distribution ratio, *K*, is defined as the ratio of concentrations in the upper phase to that in the lower.
[11] K. R. HANSON and M. ZUCKER, *J. Biol. Chem.* **238**, 1105 (1963).

large quantities of *p*-hydroxybenzyl alcohol ($K = 3{\cdot}76$) were obtained. It is uncertain whether any of this existed as such in the seed or was formed in the hydrolysis of the mustard oil.[12, 13] Examination of extracts of "Robusta" green coffee, Imperial prunes, "Rio Oso Gem" peaches and roots of *Polygala senega* likewise gave no indication of the presence of 3-*O*-sinapoylquinic acid.

The NMR spectrum of 3-*O*-sinapoylquinic acid at 100 MHz confirmed the structure in the (e) CO_2H conformation.

EXPERIMENTAL

Melting points were taken on a Kofler stage and are corrected; optical rotations were taken on a Cary 60 spectropolarimeter; NMR spectra were taken on a Varian HR-100 spectrometer.

4-*Carbomethoxysinapic Acid*

A solution of 11·2 g of 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid) in 150 ml of N NaOH was cooled to 0° and 9 ml of methyl chloroformate was added dropwise with stirring. After 45 min the reaction mixture was acidified with cold dil. HCl and the precipitate collected and dried. After recrystallization from ethyl acetate–acetone–light petroleum, the 4-carbomethoxyester melted at 165–166° (yield, 12·1 g). (Found: C, 55·5; H, 4·98. Calc. for $C_{13}H_{14}O_7$: C, 55·32; H, 5·00%).

Benzhydryl 1-*O*-*Tetrahydropyranyl*-4,5-*Isopropylidenequinate*

A solution of 29·8 g of 1-*O*-tetrahydropyranylacetonequinide[14] in 200 ml methanol was warmed on the steam bath with 150 ml 2 N KOH solution for 1 hr. The reaction mixture was evaporated *in vacuo* < 30° to about 100 ml and carefully neutralized (cooling) with 2 N HCl. The aqueous solution was further concentrated *in vacuo* and then extracted six times with equal volumes of ethyl acetate. The combined extract was washed with water, dried ($MgSO_4$) and concentrated *in vacuo* to about 100 ml. Diphenyldiazomethane was then added dropwise to a slight excess (persistent pink color). The volume was reduced to about one-third *in vacuo* and a 3-fold volume of light petroleum was added. The resulting crystals (32·5 g) were recrystallized from methyl acetate–light petroleum, m.p. 139–141°. (Found: C, 69·4; H, 7·03. Calc. for $C_{28}H_{34}O_7$: C, 69·69; H, 7·10%.)

3-O-*Sinapoylquinic Acid*

4-Carbomethoxysinapic acid (2·82 g) was allowed to stand overnight with 20 ml of $SOCl_2$ and then warmed at 60° for 1 hr. The excess $SOCl_2$ was removed *in vacuo* and the residue was dissolved in 20 ml of dry benzene. A solution of 4·82 g of benzhydryl 1-*O*-tetrahydropyranyl-4,5-isopropylidinequinate in 35 ml of benzene and 6 ml of triethylamine was added. The reaction mixture was stood at room temperature for 48 hr and then warmed at 60° for 2 hr. The solvent was removed *in vacuo*, ether and water added and the ether layer separated and evaporated to dryness. The crude product was hydrolyzed by heating on the steam bath with 100 ml of 80 per cent acetic acid for 3 hr and then diluting with 5 l. of water and refluxing for 16 hr. The solvents were removed *in vacuo* and the residue subjected to 100 transfers in a Craig countercurrent distribution apparatus (10 ml each phase: ethyl acetate–2% NaCl). The fraction in tubes 40–60 (max. tube 52) was collected and evaporated to dryness under reduced pressure. The residue was extracted with isopropanol, filtered and the filtrate again evaporated to dryness. By treating again in isopropanol, a small amount of salt was removed. Evaporation of the isopropanol in N_2 gave 0·489 g of crystalline material; recrystallized from ethanol–ether, m.p. 209–210°. (Found: C, 54·3; H, 5·65. Calc. for $C_{18}H_{22}O_{10}$: C, 54·27; H, 5·57%.) $[\alpha]_D^{27} - 43{\cdot}2°$ (EtOH); λ_{max} 327 nm ($\epsilon = 19{,}400$); NMR spectrum (d_6-dimethylsulfoxide) 100 MHz: H_4, quartet centered at 360 cps (from TMS); H_5, quartet centered at 406 cps and H_3, sextet centered at 522 cps. The coupling constants and fine structure intensities correspond to the first-order assignments of the analogous protons in chlorogenic acid.[15]

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[12] J. Barothy and H. Neukom, *Chem. & Ind.* 308 (1965).
[13] S. Kawakishi and K. Muramatsu, *Agr. Biol. Chem. (Tokyo)* **30**, 688 (1966).
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[15] J. Corse, R. E. Lundin, E. Sondheimer and A. C. Waiss, Jr. *Phytochem.* **5**, 767 (1966).