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Note

Glass capillary gas chromatographic separation of naturally occurring phloroglucinols

I. Investigation of some monocyclic acylphloroglucinol derivatives

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In connection with recent studies[1–10] on naturally occurring phloroglucinol derivatives, the separation of some analytically important monocyclic acylphloroglucinols (compounds 1–6 in Table I), 3-methylacylphloroglucinols (compounds 7–12) and their corresponding mono- and dimethyl ethers (compounds 13–29) has been investigated by high-resolution glass capillary gas–liquid chromatography (GLC). Thin-layer and paper chromatographic techniques for these compounds have been reported, but the efficiency of separation was not good for the polar substances 1–12 (see refs. 1, 11 and 12). The compounds 1–29 serve as reference compounds in analysis of the key decomposition products of naturally occurring polycyclic phloroglucinol derivatives[1–5].

EXPERIMENTAL

Investigated compounds

Table I lists the compounds studied; the synthesis and properties of compounds 1–5, 7–9, 11–25 and 29 have already been described[1–4,13–15]. The rest of the compounds were synthesized analogously[13,15–18]: isovalerylphloroglucinol (compound 6) yellow prisms, recrystallized from water, m.p. 139°, (literature value[16] 145°); 3-methyl(isobutyrylphloroglucinol) (compound 10), brownish needles, recrystallized from water, m.p. 158–159°, (literature value[17] 161–162°); 3-methyl(acetylphloroglucinol) 4,6-dimethyl ether (compound 27), brownish needles, recrystallized from water, m.p. 141°, (literature value[18] 141°); 3-methyl(propionylphloroglucinol) 4,6-dimethyl ether (compound 28), brownish needles, recrystallized from water, m.p. 133°. On mass spectrometry (MS), compounds 6, 10 and 27 exhibited M^+ peaks at *m/e* 210, 210 and 224, respectively.

Preparation of derivatives

Compounds 1–12, with three hydroxyl groups, were ethylated with diazoethane

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liberated from N-nitrosourea with potassium hydroxide. The diazoethane gas was directed into the sample (10–50 mg) in dry diethyl ether, and the mixture was allowed to stand at room temperature for 1 h before the excess of diazoethane was evaporated in a stream of nitrogen; MS was used to confirm that two hydroxyl groups have been ethylated. The GLC–MS experiments showed that by-products were not formed to any appreciable extent during the ethylation.

GLC experiments

The GLC experiments were carried out immediately after ethylation. For separation of the phloroglucinol derivatives, several glass capillary columns varying in polarity, acidity and length were constructed. Columns coated with a film of free fatty acid phase (FFAP) were found to be the most suitable for separating phenols and their ethyl ethers and were easy to prepare. In this work, capillary tubes (I.D. 0.3 mm) drawn from soda glass were etched by dry hydrogen chloride at 400° for 24 h, then deactivated with Carbowax 20M as described by Blomberg[19] and coated by the dynamic mercury-plug method of Schomburg *et al.*[20]. Columns of length 30–50 m were found to be slightly acidic[21]. A splitless-injection injection technique was used, with the samples as 1–10% solutions in diethyl ether (compounds 1–12) or chloroform (compounds 13–29). Hydrogen (2 ml/min) was used as carrier gas, and the column was temperature-programmed from 100 to 270° at 10°/min. The GLC instrument (Carlo Erba 2300) was equipped with a flame ionisation detector.

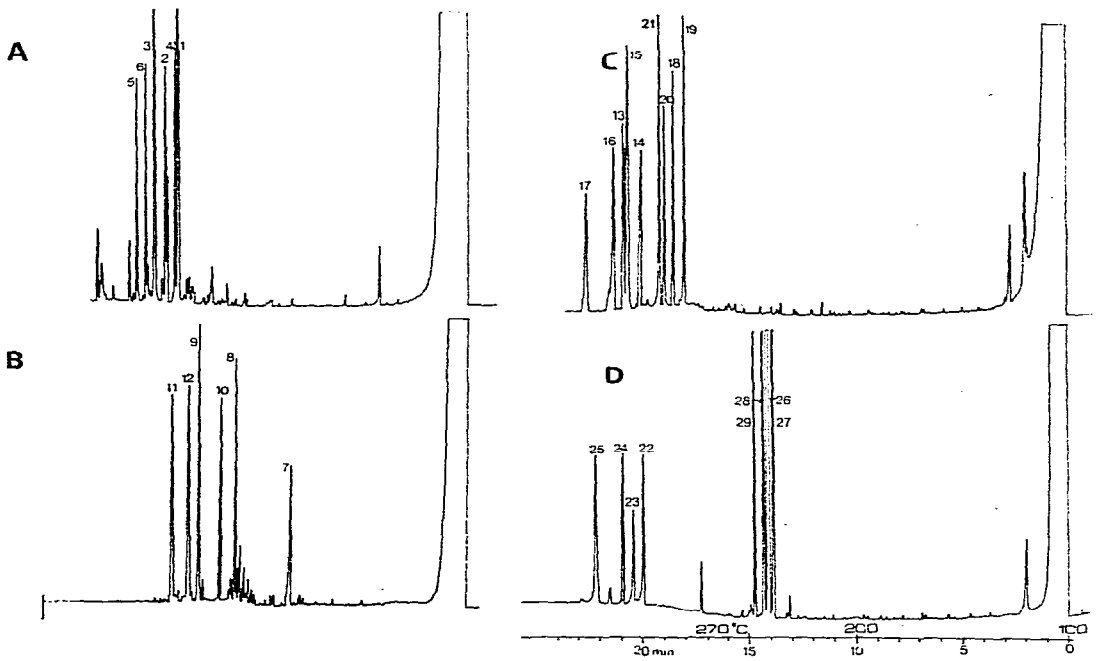
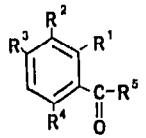


Fig. 1. Gas chromatograms obtained from (A) ethylated derivatives of compounds 1–6, (B) ethylated derivatives of compounds 7–12, (C) compounds 13–21, and (D) compounds 22–29. For chromatographic conditions, see text.

TABLE I

STRUCTURES OF THE COMPOUNDS STUDIED

The compounds listed have the following general formula:



Compound No.	R^1	R^2	R^3	R^4	R^5	*Compound name*
1	OH	H	OH	OH	CH_3	Acetylphloroglucinol
2	OH	H	OH	OH	C_2H_5	Propionylphloroglucinol
3	OH	H	OH	OH	C_3H_7	Butyrylphloroglucinol
4	OH	H	OH	OH	$CH(CH_3)_2$	Isobutyrylphloroglucinol
5	OH	H	OH	OH	C_4H_9	Valerylphloroglucinol
6	OH	H	OH	OH	$CH_2CH(CH_3)_2$	Isovalerylphloroglucinol
7	OH	CH_3	OH	OH	CH_3	3-Methyl(acetylphloroglucinol)
8	OH	CH_3	OH	OH	C_2H_5	3-Methyl(propionylphloroglucinol)
9	OH	CH_3	OH	OH	C_3H_7	3-Methyl(butyrylphloroglucinol)
10	OH	CH_3	OH	OH	$CH(CH_3)_2$	3-Methyl(isobutyrylphloroglucinol)
11	OH	CH_3	OH	OH	C_4H_9	3-Methyl(valerylphloroglucinol)
12	OH	CH_3	OH	OH	$CH_2CH(CH_3)_2$	3-Methyl(isovalerylphloroglucinol)
13	OCH_3	H	OH	OH	C_3H_7	Orthodesaspidinol B (butyrylphloroglucinol 2-methyl ether)
14	OH	H	OCH_3	OH	CH_3	Desaspidinol A (acetylphloroglucinol 4-methyl ether)
15	OH	H	OCH_3	OH	C_2H_5	Desaspidinol P (propionylphloroglucinol 4-methyl ether)
16	OH	H	OCH_3	OH	C_3H_7	Desaspidinol B (butyrylphloroglucinol 4-methyl ether)
17	OH	H	OCH_3	OH	C_4H_9	Desaspidinol V (valerylphloroglucinol-4-methyl ether)
18	OCH_3	CH_3	OH	OH	C_3H_7	Pseudoaspidinol B [3-methyl(butyrylphloroglucinol) 2-methyl ether]
19	OCH_3	CH_3	OH	OH	$CH(CH_3)_2$	Pseudoaspidinol iB [3-methyl(isobutyrylphloroglucinol) 2-methyl ether]
20	OCH_3	CH_3	OH	OH	$CH(CH_3)CH_2CH_3$	Pseudoaspidinol 2-MeB [3-methyl (2-methylbutyrylphloroglucinol) 2-methyl ether]
21	OH	CH_3	OH	OCH_3	C_3H_7	Isoaspidinol B [3-methyl(butyrylphloroglucinol) 6-methyl ether]
22	OH	CH_3	OCH_3	OH	CH_3	Aspidinol A [3-methyl(acetylphloroglucinol) 4-methyl ether]
23	OH	CH_3	OCH_3	OH	C_2H_5	Aspidinol P [3-methyl(propionylphloroglucinol) 4-methyl ether]
24	OH	CH_3	OCH_3	OH	C_3H_7	Aspidinol B [3-methyl(butyrylphloroglucinol) 4-methyl ether]
25	OH	CH_3	OCH_3	OH	C_4H_9	Aspidinol V [3-methyl(valerylphloroglucinol) 4-methyl ether]
26	OH	H	OCH_3	OCH_3	C_3H_7	Butyrylphloroglucinol 2,4-dimethyl ether
27	OH	CH_3	OCH_3	OCH_3	CH_3	3-Methyl(acetylphloroglucinol) 4,6-dimethyl ether
28	OH	CH_3	OCH_3	OCH_3	C_2H_5	3-Methyl(propionylphloroglucinol) 4,6-dimethyl ether
29	OH	CH_3	OCH_3	OCH_3	C_3H_7	3-Methyl(butyrylphloroglucinol) 4,6-dimethyl ether

RESULTS AND DISCUSSION

In Figs. 1A and 1B are shown gas chromatograms for the ethylated derivatives of compounds 1–6 and 7–12, respectively; compounds 13–21 (Fig. 1C) and 22–29 (Fig. 1D) are in the free (non-ethylated) state. It can be seen from Fig. 1 that glass capillary GLC can be successfully used to separate phloroglucinols, even if the compounds are isomers differing only in the positions of their methoxy groups or branching in their side chains. For compounds having three free hydroxyl groups, elution times are very long, even when short and strongly acidic columns are used; for such compounds, it is advisable to prepare a volatile derivative. The acyl derivatives of phloroglucinol are easily diethylated. The third hydroxyl group is strongly hydrogen bounded to the carbonyl group of the side chain and remains unethylated[22].

By using a flame ionisation detector, the compounds can bevdetected in nanogram amounts but even better sensitivity can be attained by electron-capture detection of, *e.g.*, the halogenated derivatives.

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