CHROM. 6036

Analysis of glycolic acid esters by thin-layer chromatography

Some data on the analysis of glycolic acid esters by chromatographic techniques have been published[1–3]. These mainly concerned benactyzine (2-diethylaminoethyl benzilate) present in pharmaceutical preparations. KRÁCMAR[4] used paper chromatography to detect the acidic and alcoholic hydrolysis products of glycolates.

In the present study, thin-layer chromatography was used as a rapid qualitative and semi-quantitative means for the analysis of synthesized glycolic acid esters and for checking the purity of starting materials and intermediates involved in the preparations. For instance, benzophenone is known to be formed by a hydrolytic oxidation mechanism from benzilic acid esters[5,6]. Benzhydrol sometimes appears as an impurity in benzilic acid[7]. For the analysis of cyclopentylphenylglycolates, corresponding breakdown products were synthesized and their chromatographic behaviour studied.

If the thin-layer chromatographic system for the separation of a glycolate from its impurities is known, the dry-column chromatographic techniques according to LOEV AND GOODMAN[8] can be used for purification purposes on a preparative scale.

Experimental

Materials

The basic esters of the substituted glycolic acids were prepared by transesterification of the sodium salts of the corresponding methyl esters with the appropriate alcohol[9]. The esters of 1-(2,2,2)-bicyclooctanol were prepared by the reaction of the substituted α-chloroacetyl chloride with the above-mentioned alcohol, followed by the replacement of the α-chloro atom by a hydroxyl group with silver nitrate in dioxane–water medium[10]. The preparation of the chloro-acid chlorides was accomplished by two methods. For the chlorination of benzilic acid, the modified procedure of KING AND HOLMES[11] was used, whereas the chlorination of the substituted glycolic acids was carried out according to SCHWENK AND PAPA[12]. N-Methyl-substituted hydroxypiperidine and 1-(2,2,2)-bicyclooctanol were prepared according to BIEL[13] and to ALDER AND RICKERT[14], respectively. Cyclopentylphenylcarbinol and cyclopentylphenylketone were synthesized according to NIGHTINGALE AND MAIENTHAL[15] and purified using preparative gas chromatography.

Chromatographic procedure

Both home-made and prefabricated thin layers on glass plates were used. The home-made layers, 250 µm thick, were prepared according to STAHL[16] using a slurry consisting of 25 g of silica gel (Merck, G.F.R.) and 50 ml of water, or a slurry of 35 g of alumina (Woelm, G.F.R.) and 40 ml of water applied with a Desaga applicator. Both materials contained 1% of ZS-fluorescence indicator (Riedel de Haen, G.F.R.). The plates were heated at 120° for 1 h after being air-dried for several hours. The prefabricated alumina and silica gel plates were obtained from Camag (Switzerland) and Merck, respectively, and both contained a UV (254 nm) fluorescence indicator. They were used without additional heating.

The above-mentioned compounds were applied as solutions in chloroform or methanol (0.05 M) at a distance of 2 cm from the base of the plate. The solvent front was allowed to travel 10 cm from the starting point. The plates were developed as described earlier[17].

Solvent systems

All components of the various systems were of ordinary analytical grade and were used without additional purification. The composition of the mobile phases in parts per volume were: (A) chloroform; (B) chloroform–methanol (9:1); (C) chloroform–diethylamine (9:1); (D) isobutanol–isopropanol–formic acid–water (15:15:4:10); (E) benzene–hexane–acetic acid (2:2:1); (F) cyclohexane–chloroform–diethylamine (5:4:1); (G) methanol–concentrated ammonia (99:1).

Solvent systems A–C and D–G were used on the alumina and silica gel plates, respectively.

Detection

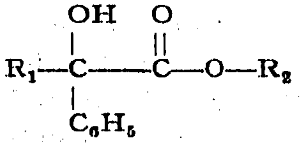
Because of the presence of phenyl groups in a number of the compounds under consideration, UV irradiation at 254 nm was used as an initial detection method. For benzophenone, this proved to be the only detection method available.

1. Iodine vapour. The plates were placed in a glass chamber saturated with iodine vapour for 15 min to develop colour, especially the nitrogen-containing compounds in a sensitive manner.

2. Potassium iodoplatinate reagent. This reagent was prepared according to GOLDBAUM AND KAZYAK[18]. With nitrogen-containing compounds it gave violet spots on a mauve background. The acids gave white spots.

TABLE I

R_F × 100 VALUES OBTAINED USING ALUMINA AND SILICA GEL AS SORBENTS FOR A NUMBER OF GLYCOLIC ACID ESTERS WITH THE GENERAL FORMULA



$R_1-C(OH)(C_6H_5)-C(=O)-O-R_2$

No.	*R_1*	*R_2*	*Solvent system*[a]						
			A	*B*	*C*	*D*	*E*	*F*	*G*
			Alumina			*Silica gel*			
1	Phenyl	3-Quinuclidinyl·HCl	22	74	60	55	5	34	43
2	Phenyl	Tropinyl·HCl	44	75	73	53	1	50	37
3	Phenyl	N-Methyl-3-piperidinyl and HCl salt	54	74	73	54	0	54	79
4	Phenyl	N-Methyl-4-piperidinyl·HCl	44	76	71	55	1	47	61
5	Cyclopentyl	3-Quinuclidinyl	43	73	71	51	5	45	56
6	Cyclopentyl	Tropinyl	38	73	71	55	2	47	41
7	Cyclopentyl	N-Methyl-3-piperidinyl	60	81	77	53	2	56	77
8	Cyclopentyl	N-Methyl-4-piperidinyl	57	80	75	55	4	49	65
9	Phenyl	2-Diethylaminoethyl	50	74	69	54	3	44	53
10	Hydrogen	3-Quinuclidinyl	10	70	43[b]	43	1	14[b]	40[b]
11	Phenyl	1-(2,2,2)-Bicyclooctyl	62	79	70	80	60	52	63
12	Cyclopentyl	1-(2,2,2)-Bicyclooctyl	65	90	74	83	78	58	73

[a] For composition, see *Experimental* section.

[b] Mean R_F values of two overlapping spots, probably resulting from diastereoisomeric compounds.

TABLE II

$R_F \times 100$ VALUES OBTAINED USING ALUMINA AND SILICA GEL AS SORBENTS FOR A NUMBER OF STARTING MATERIALS AND DECOMPOSITION PRODUCTS OF GLYCOLIC ACID ESTERS

No.	*Compound*	*Solvent system*[a]						
		A	*B*	*C*	*D*	*E*	*F*	*G*
		Alumina			*Silica gel*			
13	Methyl benzilate	63	73	74	82	54	61	—
14	Methyl cyclopentylphenylglycolate	68	81	82	78	67	63	—
15	3-Quinuclidinol	0	25	18	30	2	3	26
16	Tropine	0	39	28	32	0	8	21
17	N-Methyl-3-piperidinol and HCl salt	7	48	30	26	0	17	59
18	N-Methyl-4-piperidinol	4	47	25	26	1	12	52
19	2-Diethylaminoethanol	0	70	51	38	33	0	34
20	1-(2,2,2)-Bicyclooctanol	33[b]	50[b]	55[b]	86	57	45	67
21	Benzophenone	64	87	75	87	64	59	72
22	Benzhydrol	32	70	66	86	50	49	71
23	Cyclopentylphenylcarbinol	36	75	66	86	54	53	71
24	Cyclopentylphenylketone	64	89	76	87	72	60	72
25	Mandelic acid	0	0	0	61	5	0	51
26	Benzilic acid	0	0	3	78	31	0	—
27	Cyclopentylphenylglycolic acid	0	0	8	81	48	1	—

[a] For composition, see *Experimental* section.
[b] Weak detection with potassium iodoplatinate (white spots).

3. Cerium sulphate–sulphuric acid reagent[10]. An intense red colour was obtained with benzilic acid and its esters. A greyish colour was observed when 1-(2,2,2)-bicyclooctanol, cyclopentylphenylglycolic acid, mandelic acid and their esters were present. The reagent gave yellow and brown coloured spots with benzhydrol and cyclopentylphenylcarbinol, respectively. The reagent could not be used on alumina plates as a chemical reaction took place with the adsorbent.

Discussion

The $R_F \times 100$ values of a number of glycolic acid esters, starting materials and decomposition products are summarized in Tables I and II.

When comparing structurally related compounds, it can be seen that the $R_F \times 100$ values generally increase when a phenyl group is replaced by a cyclopentyl group, *e.g.*, compounds 1 and 5. A similar result is observed when a C=O group is substituted for a C–OH group, as illustrated by comparison of benzophenone (21) and benzhydrol (22). These effects may easily be explained as a result of the lower polarity of cyclopentyl and C=O groups when compared with phenyl and C–OH groups, respectively.

It can be seen from Table I that system A on alumina and system G on silica gel differentiate sufficiently in terms of $R_F \times 100$ values within the group of glycolates to permit an identification. These systems may be used advantageously to analyse these compounds for unknown but structurally related impurities.

In order to separate simultaneously the acidic and alcoholic hydrolysis products and the basic glycolates, solvent systems D and E or F on silica gel can be used. In this case, systems E and F are especially effective for separating benzilic acid (20), benzophenone (21) and benzhydrol (22). On alumina layers, a comparable result can be obtained by using a combination of systems A and B or C.

Small quantities of benzophenone and cyclopentylphenylketone were observed as impurities in stored aqueous solutions of benzilic acid and cyclopentylphenylglycolic acid, respectively. Detection by UV irradiation at 254 nm both on alumina and silica gel layers proved to be sufficiently sensitive for both ketones, particularly benzophenone.

On one occasion, a small amount of benzophenone (21) was detected as an impurity in 1-(2,2,2)-bicyclooctyl benzilate (11). It can be seen from Tables I and II that the $R_F \times 100$ values of these compounds obtained for different solvent systems show only minor differences. Benzene proved to be a more selective mobile phase in this case (R_F for benzophenone is 0.46, R_F for 1-(2,2,2)-bicyclooctyl benzilate is 0.29). Consequently, a silica gel–benzene system was used to purify the glycolate by the dry-column chromatographic technique according to Loev and Goodman[8].

The detection limits of the glycolates and the impurities that occasionally appear were found to be approximately (5–25) × 10^{-3} μmole. It follows that it is generally possible to detect impurities down to 1–5% when spotting amounts of 0.5 μmole.

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