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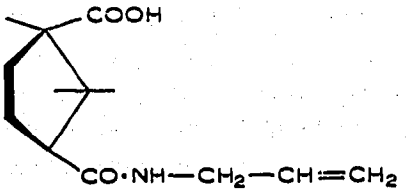
Thin layer chromatography of the α and β structural isomers of N-allyl-*dl*-camphoramic acid*

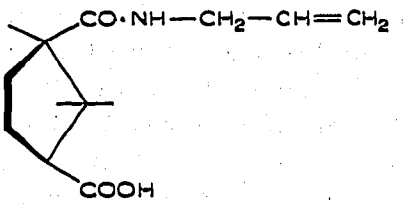
During the preparation of N-allyl-*dl*-α-camphoramic acid (I) and N-allyl-*dl*-β-camphoramic acid (II) in our laboratory[1] a method for detecting the two isomers and very small amounts of impurities was necessary. As no method has been reported in the literature, a method which could be used for this purpose was developed.

In the first place an attempt was made to find a suitable spraying agent capable of locating the spots of colourless substances. DUNCAN AND PORTEOUS[2] and REID AND LEDERER[3] have used a solution of methyl red and bromothymol blue (acid indicator) for the location of aliphatic carboxylic acids. This also proved suitable for our work and had a sensitivity up to 2 μg; the spots appeared red against an orange background. To locate the unreacted product (allylamine), a 2 % solution of iodine in methanol was used. This was suitable for locating 0.5 μg of compounds containing a double bond, which appear as brown spots against a yellow background. The methods of MARCUSE[4], BRAUN AND GEENEN[5], PASTUSKA[6], and PASTUSKA AND PETROWITZ[7] were tried but proved unsuccessful for the present purpose.

The solvents used for thin layer chromatography (TLC) were *n*-butanol saturated with water in case of a mixture of I and II, and *n*-butanol saturated with 7 % ammonia in case of I and II together with camphoric acid. All the systems used are single-phase systems at 25°, which greatly facilitates their preparation and use.



I



II

Experimental

Reagents. The following reagent grade chemicals were used without further purification: acetone, 28 % ammonia, *n*-butanol, iodine, methanol, camphoric acid, camphoric acid anhydride, Kieselgel G (Merck).

Apparatus. The tank used for the TLC was a conventional 24 × 22 × 8 cm jar. The kieselgel was spread on 20 × 20 cm plates as described by NYBOM[8].

Procedure. A layer of Kieselgel G, 0.25 mm was applied to the plates which were activated by heating at 100° for 30 min. The plates were spotted with acetone solutions of the materials, each spot containing 5–20 μg of material. No equilibration time was necessary and the temperature was maintained at 25°. It required 2 h for the solvent to travel a distance of 15 cm. After development the plates were dried using a hair dryer placed at a distance of 30 cm for 15 min and then sprayed with the locating agent.

* Abstracted from a thesis by R. G. HENEIN in fulfilment of the requirements for the degree of Doctor of Philosophy, Institute of Pharmaceutical Technology, Medical University, Budapest.

Results and discussion

The resolving power of the system is very sensitive to the amount of water present, hence the system is composed of *n*-butanol–water (100:20) *i.e.* the amount of water required to saturate the *n*-butanol at 25°. Activation of the plates before use, and storage in a dry chamber until used is also necessary. The solvent system was found suitable for I and II, and for camphoric acid anhydride and allylamine. The R_F values obtained are shown in Table I.

TABLE I

R_F VALUES OBTAINED USING THE TWO SOLVENT SYSTEMS AT 25°

Substance	*n-Butanol–water (100:20)*	*n-Butanol–7 % ammonia (100:20)*	
		1 run	*3 runs*
I	0.80	0.31	0.50
II	0.58	0.30	0.40
Camphoric acid anhydride	0.89	0.17	0.21
Camphoric acid	*	0.08	0.12
Allylamine	0.08	0	0

* Cannot be determined as it tails.

With the above solvent system it is possible to verify the absence or presence of camphoric acid; but when it is present, it renders the system questionable as it tends to locate itself between I and II and tails. A large number of solvents were tested including mixtures of *n*-butanol and acetic acid, *n*-butanol and pyridine, acetone and propanol, benzene and *n*-butanol. Little success was obtained until aqueous ammonia was introduced into the solvent or the spots were applied as their ammonium salts. The most suitable system was *n*-butanol saturated with 7 % ammonia (100:20 at 25°), found which clearly showed the presence of I and II and the acid, but I and II were situated so close together that the boundaries of the spots were not clear. Using the formulas of JEANES AND WISE[9] and THOMA[10] for predicting the optimum number of runs required to separate them, we found that at least two consecutive runs are required. A drying time of 30 min was allowed between runs. The R_F values obtained after one and three runs are given in Table I.

The *n*-butanol–water system is capable of resolving amounts up to 1000 μg and hence can be used as a separation method.

Examination of the reaction mixture of WOOTON[11] showed that both I and II are formed. On recrystallisation from acetone it was found that the crystals are pure I; II being more soluble and present in a lower concentration remains in the mother liquor. The method used by WENDT AND BRUCE[12] for the preparation of I also gave rise to II, which remained, however, in the aqueous methanol during recrystallisation.

The method can also be used to estimate the amount of I in II or *vice versa*. This was carried out by spotting concentrations rising from 5 to 100 μg (twenty spots) and by finding the concentration at which one spot only can be visualised and the next concentration at which the second spot appears. Knowing the sensitivity

of the iodine indicator we could thus calculate with reasonable accuracy the composition of mixtures of I and II.

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A method for quantitative thin-layer chromatography using the flask combustion method

Generally, an extraction or a densitometric method is used for quantitative thin-layer chromatography. Previously, we reported another useful method whereby a gas chromatographic technique of elementary analysis is used for the determination of organic substances containing nitrogen[1]. On the other hand SOEP[2,3] has reported the determination of fluorine on paper chromatograms by means of the flask combustion method. In the present paper, we describe a convenient method for the determination of organic substances containing halogens on thin-layer chromatograms by the flask combustion method[4,5].

Apparatus and procedure

The combustion flask consists of a 500 ml Erlenmeyer flask and a sample holder or an electrode[4,5]. Chromatoplates (5 × 20 cm) were layered with Kiesel Gel H (Merck AG, Darmstadt), Wako Gel B-10 (Wako Chem., Osaka), Aluminium Oxide G (Merck), Cellulose TLC (Serva, Heidelberg) or Silica Gel H-F, in the usual manner (0.2–0.3 mm thickness). Silica Gel H-F was prepared by mixing 80 ml of 0.003 % aqueous uranine (Fluoresceine sodium) solution with 32 g of Kiesel Gel H.

Samples (*ca.* 400 μg) dissolved in ethyl acetate, benzene or methanol were