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# SOME ASPECTS OF THIN-LAYER CHROMATOGRAPHY AND OF ITS APPLICATION IN PHARMACOGNOSY

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SUMMARY

Some applications of TLC in the pharmaceutical research laboratory are given. Separations of steroids from *Solanum paniculatum*, metabolites of some nitroimidazole derivatives and some pharmacognostic investigations are discussed in detail.

## INTRODUCTION

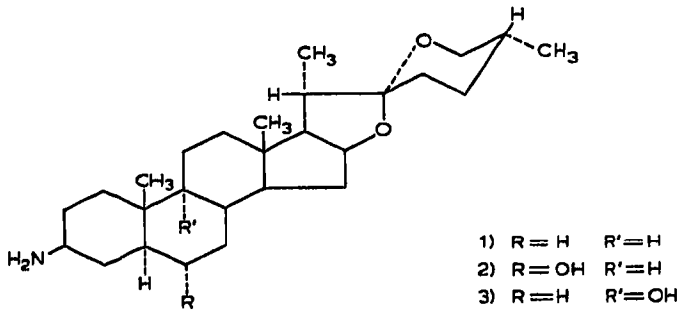
I am sure that one of the reasons why thin-layer chromatography (TLC) has gained prompt and widespread acceptance in many laboratories is its remarkable adaptability to the modifications and technical refinements that each laboratory or individual investigator is often forced to make in order to solve certain immediate problems.

From the investigations carried out in our research institute, concerning the metabolism of drugs and their pharmacognosy, I would like to give a few examples where TLC was particularly succesful.

## DRUG METABOLISM

In chromatographic studies of synthetic and metabolic derivatives of aminospirostanes, isolated in the *iso*- form from the roots of *Solanum paniculatum* (Fig. 1), we were able to confirm certain results already partly known from the literature. We found that the introduction of a double bond in the 5- position of a steroid group decreases the polarity of this group and that the 6$\beta$- and 7$\beta$-hydroxyl derivatives are less polar than the 6$\alpha$- and 7$\alpha$-hydroxyl derivatives, respectively. Also, the 3$\beta$-azide compounds are less polar than their 3$\alpha$-azide counterparts (Fig. 2). Although our attempts to separate the *iso*- forms (25$\alpha$ or 25R) of these compounds from the neo-forms (25$\beta$ or 25S) were unsuccessful, comparative studies of other *iso*- and neo-steroid forms showed that whenever separation is possible, the *iso*- are less polar than the neo- forms. These observations represent a useful criterion for the discrimination of various isomers; they are confirmed in the literature.

Investigations on nitroimidazole derivatives endowed with antiprotozoan properties, and more particularly trichomonacidal activity, have shown that whereas microanalytical results were consistently in agreement with theoretical values, the




(25R)-3β-Amino-5α,22 α,O-spirostan and 6α-/9α-hydroxyderivatives from *Solanum Paniculatum* roots.

Fig. 1. General structural formula for aminospirostanes isolated from *Solanum paniculatum* roots.

microbiological activity varied from one preparation to another. These products are obtained by reacting the sodium salt of nitroimidazole with ethyl morpholine chloride (see Fig. 3). As a result of TLC, we were able to separate two isomers whose polarity, in a neutral medium, was inverted when basic eluents were subsequently used. Evidently the two compounds had different basicities, and the more strongly basic of

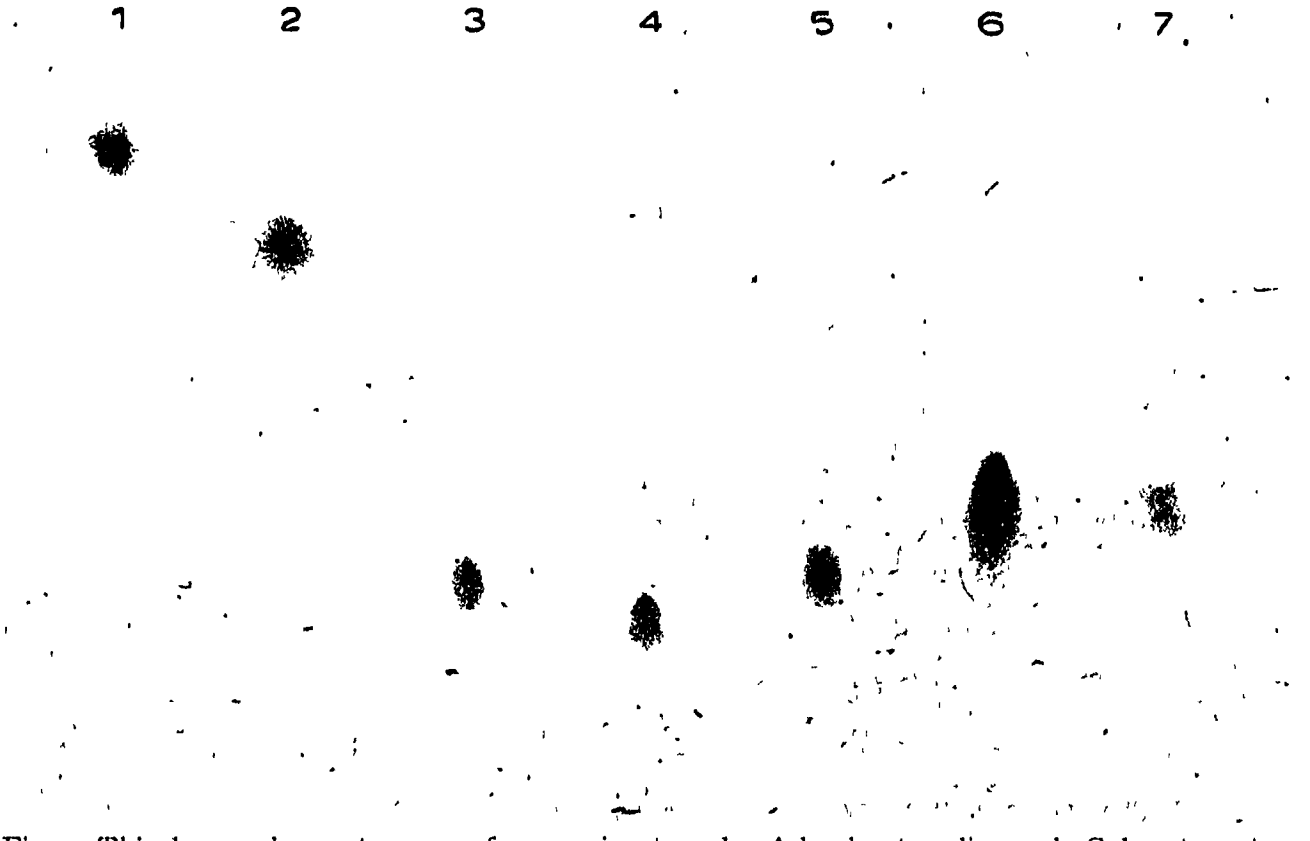



Fig. 2. Thin-layer chromatogram of isomeric steroids. Adsorbent: silica gel Solvent system: chloroform–methanol–conc. ammonia (100:15:1.5). Detection vanillin–sulphuric acid reagent. 1 = 3β-amino, $\Delta^5$, 2 = 3β-amino; 3 = 3α-amino, 6α-ol, 4 = 3β-amino, 6α-ol, 5 = 3β-amino, 6β-ol, 6 = 3β-amino, 7β-ol, 7 = 3β-amino, 7α-ol.

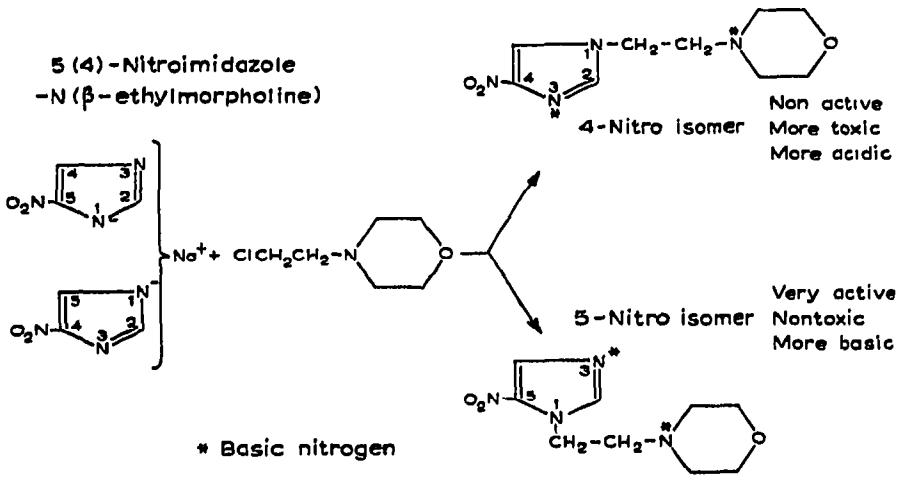



Fig. 3. Structural formulae for the 4- and 5-nitro isomers of imidazole-N(β-ethylmorpholine).

the two migrated less in a neutral medium but faster in a basic medium. The following reactions were carried out on the plate itself: reduction with titanium trichloride followed by diazotization with amyl nitrite and coupling with naphthylethylene-

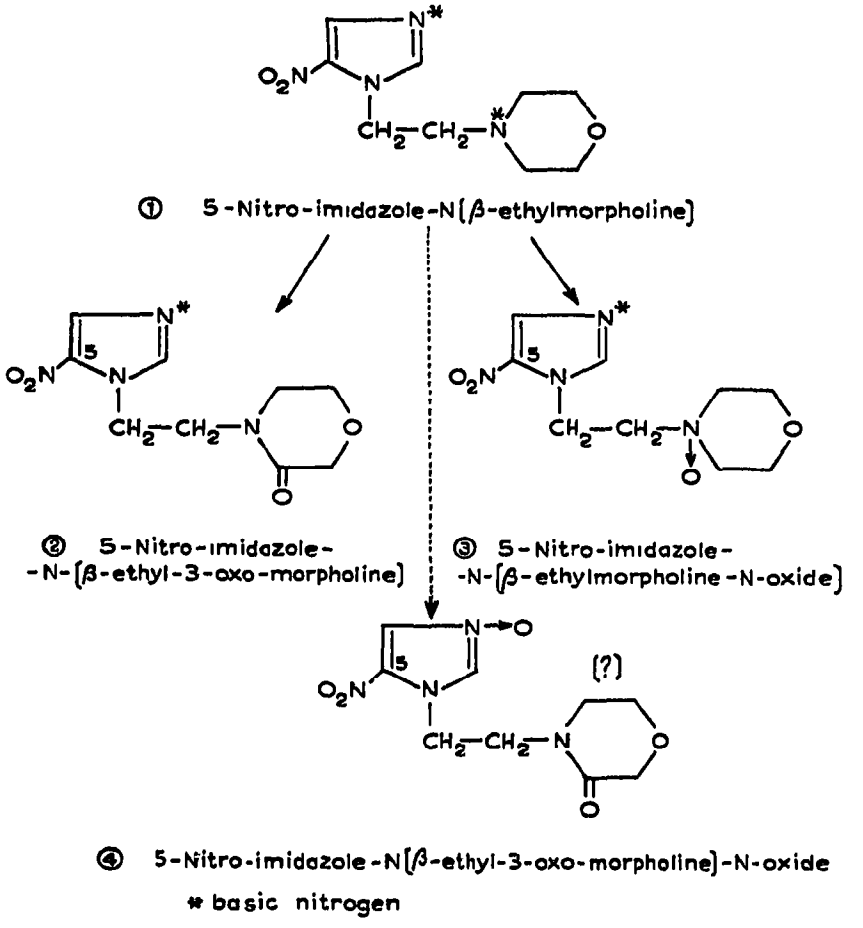



Fig. 4. Structural formulae for the metabolic derivatives of 5-nitro-imidazole-N(β-ethylmorpholine) found in humans.

diamine. This showed that both substances contained a nitro group. Even before we proceeded to separate the mixture on a column and carry out spectrometric analyses, we guessed that we were dealing with 4- and 5-nitro-imidazole-N (β-ethylmorpholine), respectively, and that the isomer with the lower $R_F$ in the basic eluent was the 4-nitro compound, its characteristics being more acidic as a result of the greater electron-withdrawing power of the 4-nitro group.

Following oral administration to humans of 5-nitro-imidazole-N(β-ethylmorpholine), three metabolites were recovered in the urine (Fig. 4); these, in a basic TLC system, were distributed on the plate according to their basicity. (See Fig. 5.)

Isolation and determination of structures 2- and 3-, as shown in Fig. 4, provided confirmation of the polarity/acidity scale, and also were in accordance with the literature data that the human metabolism of nitroimidazole derivatives is prevalently oxidative. The 4th structure has not been definitely determined as yet because of the

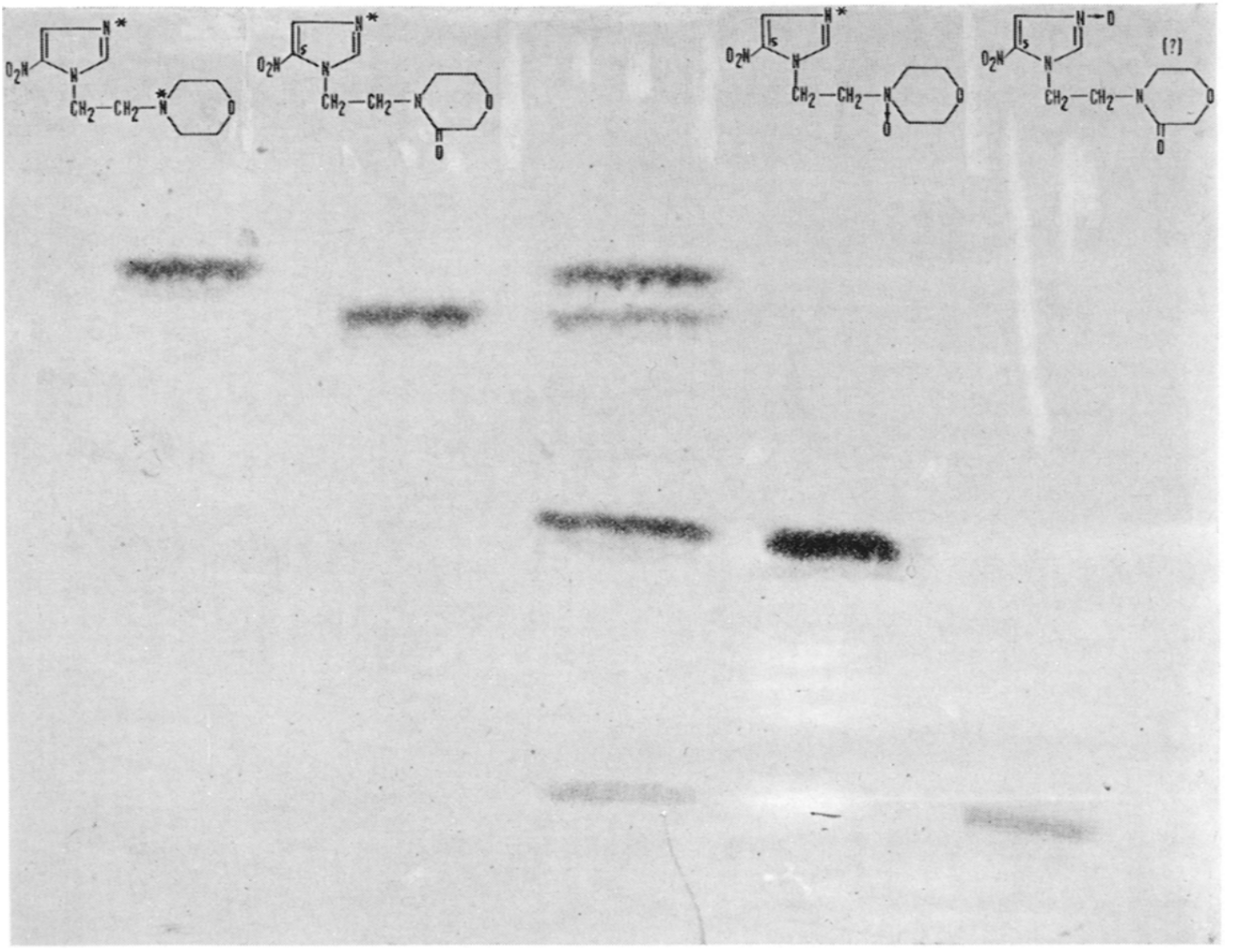


Fig. 5. Chromatogram of the metabolic derivatives of 5-nitro-imidazole-N(β-ethylmorpholine) found in humans. Adsorbent: silica gel. Solvent system: chloroform–methanol–conc ammonia (70:20·5). Detection: sprayed first with a 50% solution of phenylacetonitrile in acetone and then with a saturated solution of trimethylbenzylammonium hydroxide in methanol, yielding brown spots.

difficulties encountered in its isolation and purification; however, considering that its basicity is practically nil, we are inclined to believe that it is compound 4 in Fig. 4, as the amidic nitrogen is not basic and both the basic nitrogens are substituted.

## PHARMACOGNOSY

The many difficulties confronting the pharmaceutical chemist, including the identification of different chemical species in plants where two botanically identical plants may contain different chemical substances, for instance, have promoted the introduction of chromatographic techniques in pharmacognosy. One advantage of TLC is the availability of many more methods of detection than are available with other procedures.

Thus the reaction by which catechins react with aldehydes in the presence of strong acids to yield triphenylmethane dyes was utilized to detect catechinotannins selectively on thin-layer chromatograms. Developed chromatograms were sprayed with a saturated solution of vanillin in 50% phosphoric acid and then heated for a few minutes at 110°. Cherry-red spots appear in the presence of catechino-derivatives; gallic and ellagic acid compounds do not react. The sensitivity is about 0.5 $\mu$g (Fig. 7). On the other hand, another reagent, Fast Blue Salt B, reacts with all three types of tannin. In the pharmaceutical industry where the requirements are reflected by that

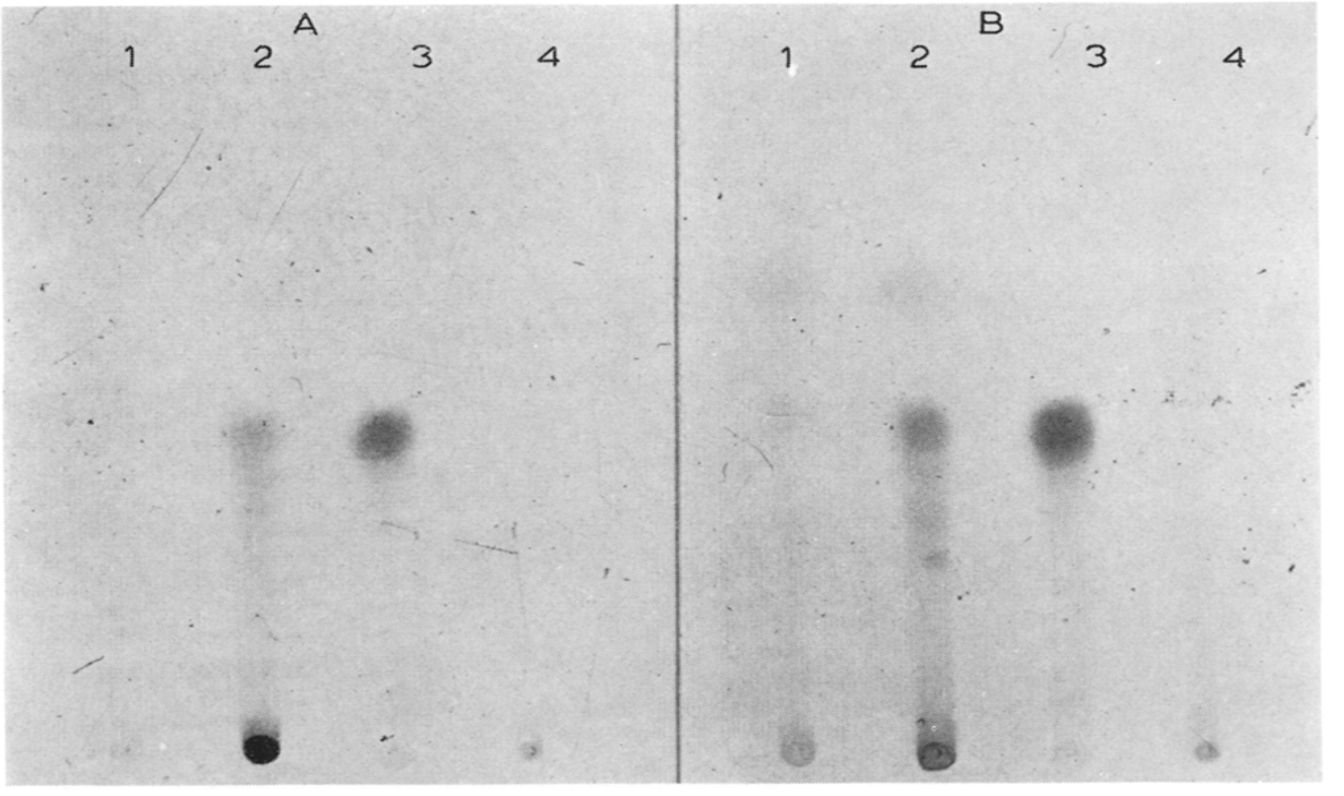



Fig. 6. Chromatogram of catechinotannins using silica gel as adsorbent and chloroform–ethyl acetate–formic acid (5:4:1) as the solvent system. Detection: A = 1% vanillin in sulphuric acid; B = Fast Blue Salt B reagent. 1 = Gallotannin, 2 = *Polygonum bistorta*; 3 = catechin; 4 = ellagic acid.

Fig. 7. Photograph of *Helleborus niger*.

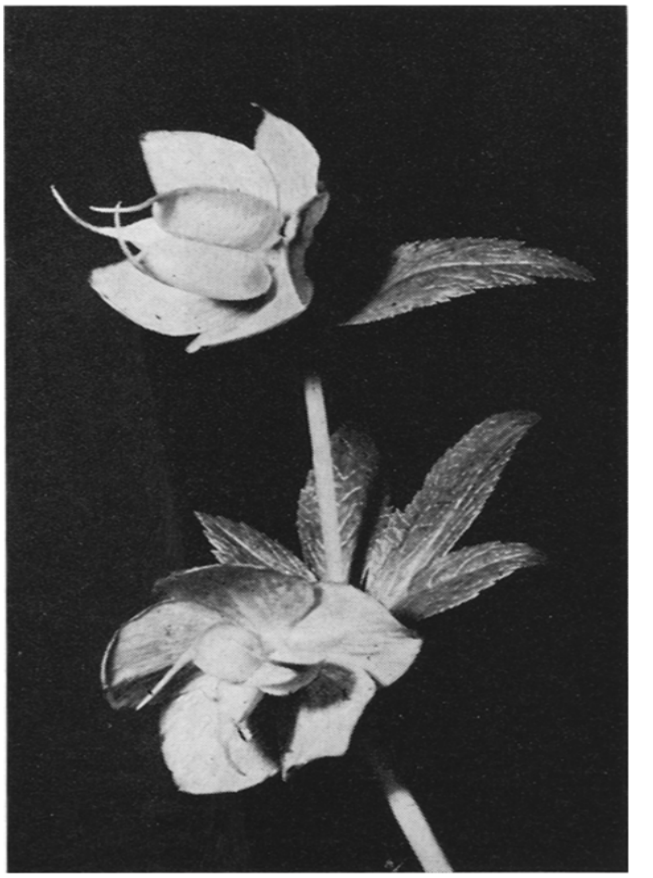


Fig. 8. Photograph of *Helleborus viridis*.

of the pharmacopoeia, the pharmacognostic problem has therepeutic and economical implications. The following examples illustrate this point. KARRER's[1] investigations showed that *Helleborus niger* (Fig. 7), gathered in central and northern Europe contains hellebrin, one of the most potent cardiotonics in existence, whereas the similar species, *Helleborus viridis* (Fig. 8), is practically devoid of it. In specimens gathered in Italy, however, we found that the opposite was true (Fig. 9).

Although *Aloe vera L.* (Curaçao aloe) is reputed to be the species containing most aloin, and is therefore used for its extraction, there are specimens of this plant that contain no aloin at all (Fig. 10).

The *Bulbus Scillae* were once divided into a wide variety, which contained cardiotonic scillarens, and a red variety, which contained inactive scillirosides. In fact, many varieties of white squill contain neither scillaren A nor proscillaridin A, and many varieties of red squill contain rather little scilliroside (Fig. 11).

Another example of current interest concerning the chemical composition of different botanical species is that of valerian. The study of the chemistry of this drug seems to have received a new impetus from the discovery of the valepotriates (Fig. 12) which are thought to represent the sedative principles of this plant. Thanks to TLC, we were able to detect (and later to isolate in a column) the same esters from other *Valerianaceae*, such as *Fedia cornucopiae*, a typically Mediterranean

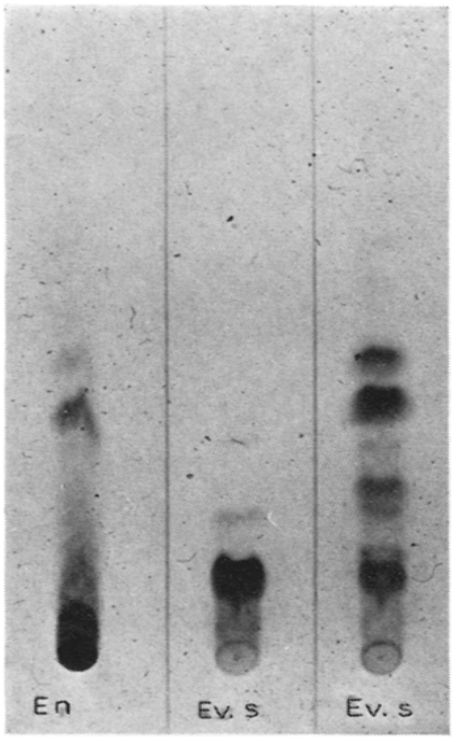



Fig. 9. Chromatogram of hellebrin in *Helleborus niger* and *Helleborus viridis*. Left to right: En = *Helleborus niger* extract; Ev5 = hellebrin; Evs = *Helleborus viridis* extract. Adsorbent: silica gel. Solvent system: chloroform–methanol–conc. ammonia (100:20:2). Detection: vanillin–phosphoric acid reagent.

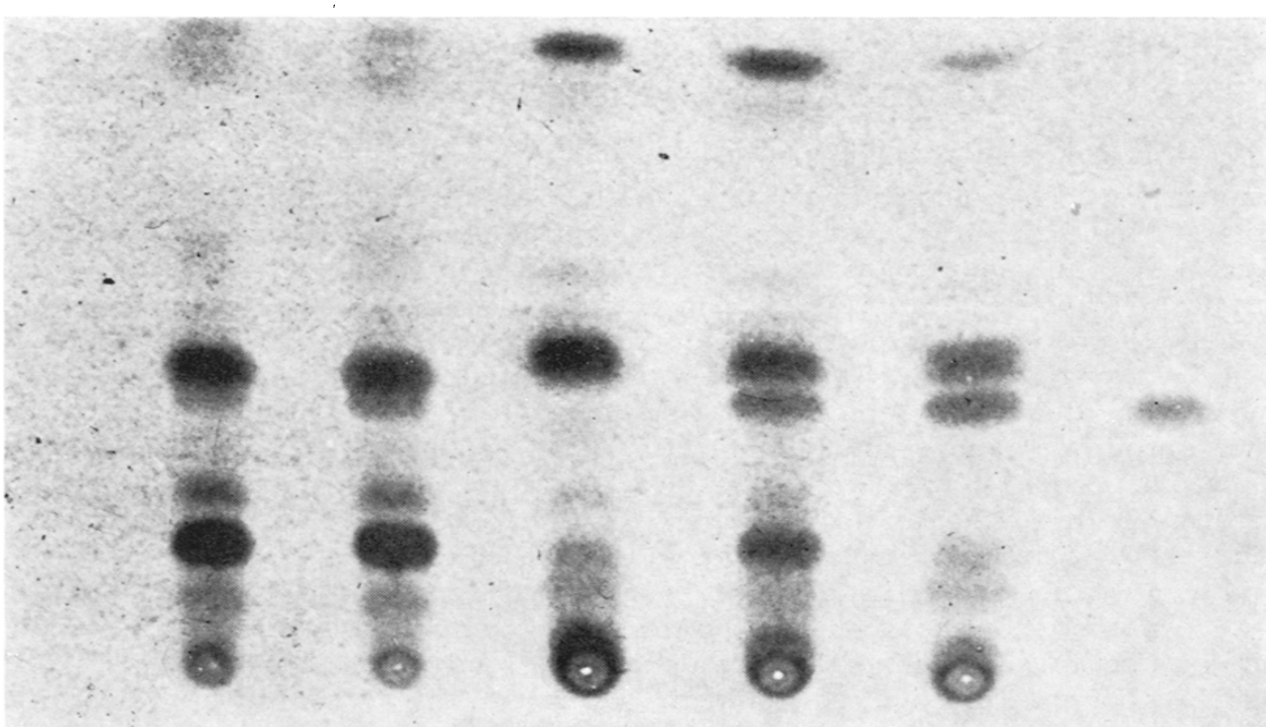


Fig. 10. Chromatogram of species of *Aloe*. From left to right: aloin standard, Indian Aloe, Curaçao Aloe containing aloin, Curaçao without aloin, and Kap Aloe (last two ). Adsorbent: silica gel. Solvent system: ethyl acetate–ethanol–water (100:13.5·10). Detection· Fast Blue Salt B reagent.

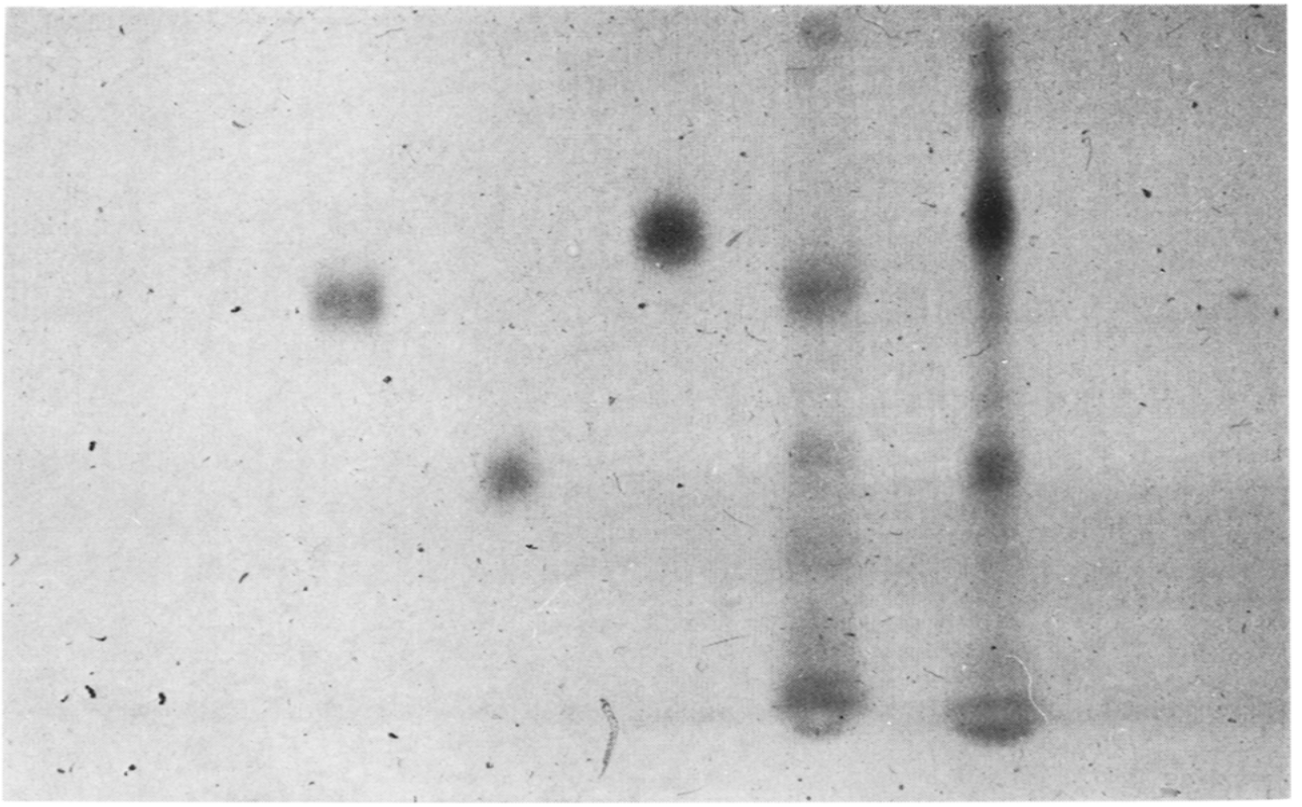


Fig. 11 Chromatogram of varieties of *Scilla*. From left to right: scilliroside, proscillaridin A, scillaren A, and 2 scilla extracts. Adsorbent: silica gel. Solvent system: ethyl acetate–ethanol–water (100:13.5:10). Detection 25% $SbCl_3$ in chloroform.

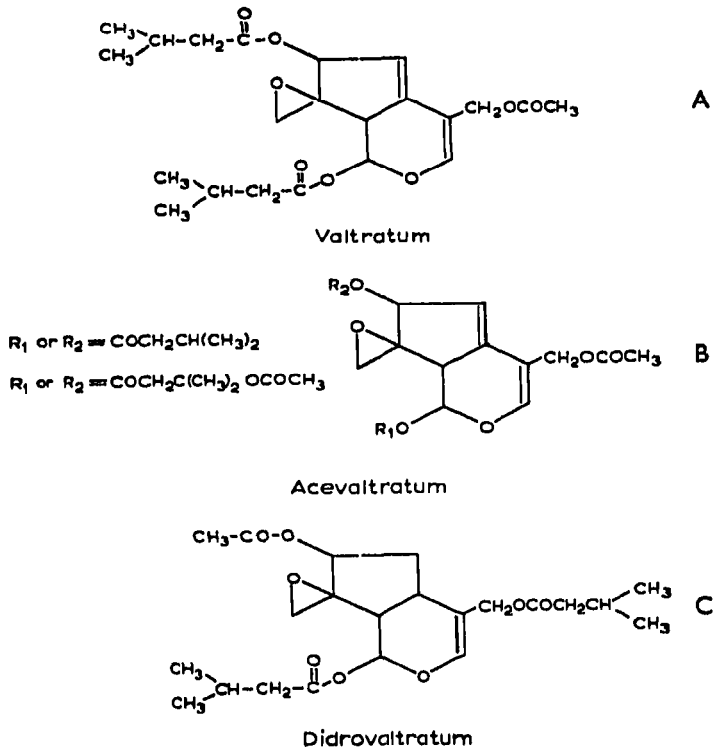



Fig. 12. Structural formulae of *Valepotriates*.

plant; on the other hand, many samples of European valerian are devoid of valepotriates (Fig. 13).

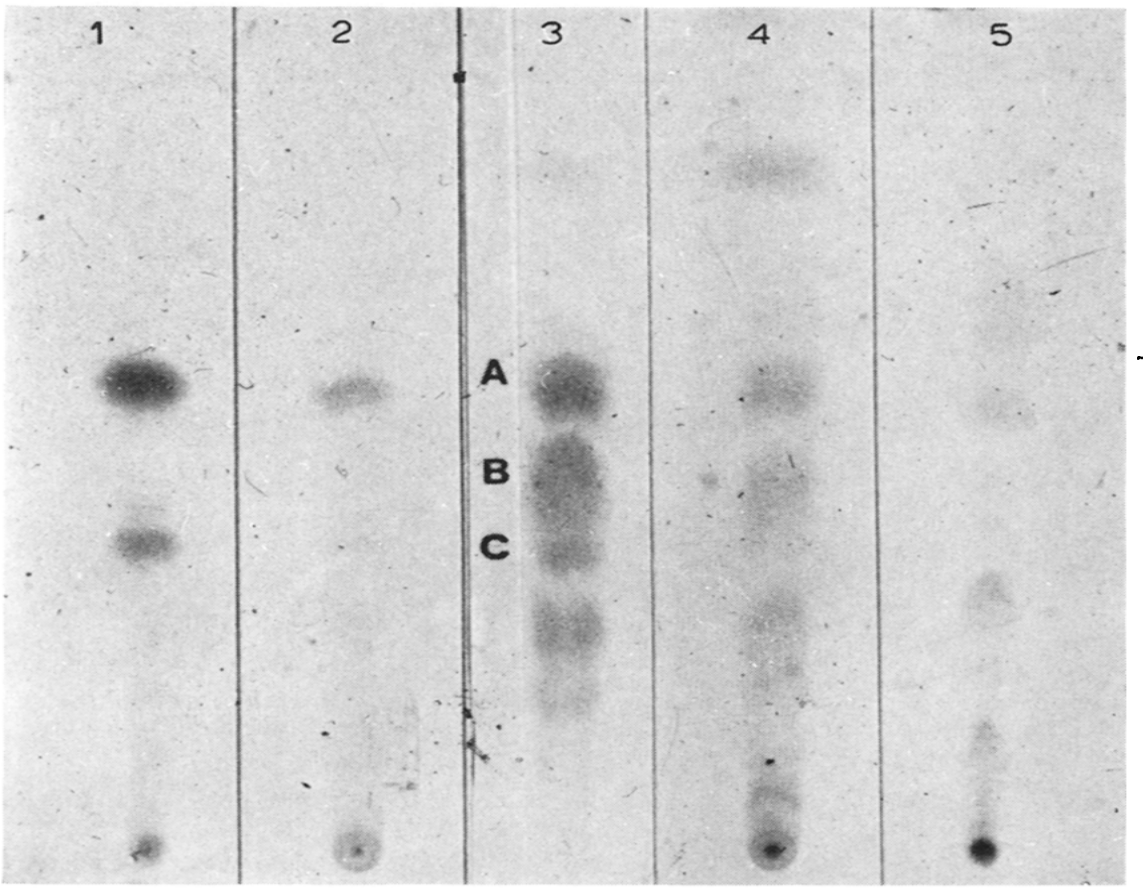



Fig. 13. Chromatogram of valepotriates detected in some *Valerianaceae*. Adsorbent: silica gel Solvent system: *n*-hexane–ethyl acetate (73:27). Detection: vanillin–sulphuric acid reagent. 1 = *Centranthus ruber* roots; 2 = *Fedia cornucopiae* plant; 3 = valepotriates mixture, A = valtratum, B = didrovaltratum, C = acevaltratum; 4 = *Valeriana wallichii* root-stock (India); 5 = *Valeriana officinalis* roots.

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REFERENCE

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