Solvent Systems for Silica-gel Column Chromatography of Indole Derivatives

An earlier paper[1] described a method for separating a number of indole derivatives and auxin-like substances in plant extracts, using silica-gel partition columns and petroleum ether: *n*-butyl alcohol solvents used with a step-wise elution technique. Further investigations, the results of which are reported here, have extended this technique to include a number of other useful solvent mixtures.

Most eluting mixtures consisted of a more polar solvent that had been saturated with 0·5 M formic acid, and a non-polar one. A few consisted only of the acid-saturated polar constituent. The column, described in detail elsewhere[1], was prepared by hydrating 8·0 g of silica gel with 5·0 ml. of 0·5 M formic acid, then slurring the hydrated silica gel with the eluting solvent and pouring into a glass tube 14 mm internal diameter. The column was about 13 cm high after it was packed by applying air pressure from a hand squeeze bulb. Approximately 5×10^{-7} moles of each indole derivative were chromatographed and collected in 3-ml. fractions on a fraction collector. When mixtures of indoles were chromatographed it was often necessary to use a step-wise elution technique in which increasing concentrations of the more polar constituent were utilized in order to obtain a satisfactory separation. Each fraction was taken to dryness in the test tube in which it was collected *in vacuo* by use of a rotating evaporator (Rotary Evapo-Mix, manufactured by Buchler Instruments, Inc., Fort Lee, New Jersey). The indoles were afterwards detected with the Gordon–Weber modification of the Salkowski reagent[2].

Most of the solvent mixtures used *n*-hexane as the non-polar phase. Hexane does not cause column cracking at elevated laboratory temperatures as does the petroleum ether, b.p. 30°–60°, used in earlier work[1]. The hexane solvent mixtures are listed in Table 1, and their chromatographic properties with seven indoles of widely varying solubility characteristics in Table 2. As a group the alcohol-hexane mixtures were the most versatile. At a given concentration they could elute a particular indole more readily than any of the other solvents. With one or two exceptions they were able to separate the seven indoles listed in Table 2. A definite inferiority of one alcohol over another one was not established, with the exception of hexyl alcohol, and possibly *n*-butyl alcohol, where their higher boiling points may be objectionable. The ester-hexane mixtures were intermediate in their ability to elute the indoles. They generally effected elution and separation of all the indoles except tryptophan and tryptamine. All but two of these esters have boiling points appreciably lower than the alcohols. This is a distinct advantage in laboratories not equipped for efficient evaporation of liquids from test tubes. Butanone-hexane was a slightly stronger eluting solvent than the esters; it could elute tryptophan and tryptamine. Chlorinated hydrocarbon-hexane mixtures were least satisfactory as a group. Methylene chloride, chloroform, and 1,2-dichloroethane in high concentrations were able to elute all the indoles of Table 2 except tryptophan and tryptamine. Carbon tetrachloride and trichloroethylene were able to efficiently elute only ethyl indoleacetate and indoleacetonitrile, and would thus appear to have little value as eluting solvents for silica-gel column chromatography of indole compounds. In no case did any of the chlorinated hydrocarbons resolve ethyl indoleacetate and indoleacetonitrile.

The choice of the non-polar constituent does, of course, markedly affect the elution pattern. Benzene with *n*-butyl alcohol, for example, eluted ethyl indoleacetate and indoleacetonitrile much more readily than did *n*-hexane. Even closely related non-polar solvents could give different results; *n*-pentane and petroleum ether (b.p. 30°–60°) with 0·2 per cent *n*-butyl alcohol were able to effect separation of ethyl indoleacetate and indoleacetonitrile, whereas *n*-hexane in similar or any other likely concentration was unable to do so.

Table 1. Some Solvents used for the Silica-gel Column Chromatography of Indole Derivatives

n-Hexane : methylene chloride	*n*-Hexane : ethyl acetate
n-Hexane : chloroform	*n*-Hexane : *n*-propyl acetate
n-Hexane : carbon tetrachloride	*n*-Hexane : methyl propionate
n-Hexane : 1,2-dichloroethane	*n*-Hexane : ethyl propionate
n-Hexane : trichloroethylene	*n*-Hexane : *n*-butyl alcohol
n-Hexane : 2-butanone	*n*-Hexane : *sec*-butyl alcohol
	n-Hexane : isobutyl alcohol
n-Hexane : methyl formate	*n*-Hexane : *t*-amyl alcohol
n-Hexane : ethyl formate	*n*-Hexane : *n*-hexyl alcohol
n-Hexane : methyl acetate	

Table 2. Ability of Chromatography Solvents to Elute Indole Derivatives from Silica Gel Columns

	Concentration of solvent in *n*-hexane required for elution		
	Chlorinated hydrocarbons	Esters and 2-butanone	Alcohols
Ethyl indole-3-acetate	50–100%	3–5%	0·5–1%
Indole-3-acetonitrile	50–100%	3–5%	0·5–1%
Indole-3-butyric acid	100% to not eluted	25%	5–10%
Indole-3-acetic acid	100% to not eluted	25%	5–10%
Indole-3-acetamide	100% to not eluted	50%	25–50%
Tryptophan	not eluted	100% to not eluted	100%
Tryptamine	not eluted	100% to not eluted	100%

Using the solvent systems above, the indoles were generally eluted by 50 ml. or less of solvent and came off the column as sharp peaks in volumes of about 6–15 ml. Substances said to be not eluted did not come off the column when quantities of 100–200 ml. of the solvents were used.

With one or two exceptions all the solvent systems eluted the seven indoles in the same order, if indeed they eluted and separated them at all. That order was ethyl indoleacetate, indoleacetonitrile, indolebutyric acid, indoleacetic acid, indoleacetamide, tryptophan, and tryptamine.

Full details of the solvent systems used have been purposely omitted since the substances to be dealt with in a particular problem are not likely to be exactly those used here. The information given should be sufficient, however, to guide the investigator towards a wise choice for his own circumstances.

This work was carried out while the author was a member of the staff of the Department of Pomology, New York State Agricultural Experiment Station, Cornell University, Geneva.

Loyd E. Powell

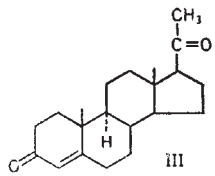
Department of Pomology,
Cornell University,
Ithaca, New York.

[1] Powell, Loyd E., *Plant Physiol.*, **35**, 256 (1960).
[2] Gordon, S. A., and Weber, R. P., *Plant Physiol.*, **26**, 192 (1951).

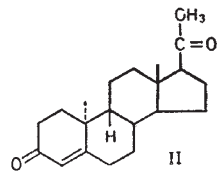
Metabolism of some Progestationally Active 9β,10α-Steroids in Man

In 1960 Reerink *et al.*[1] announced a new class of hormonally active steroids named 'retro-steroids'. This class is characterized by the β-position of the C-9-hydrogen atom and the α-position of the C-10-methyl group.

In clinical trials two members of this class, 6-dehydro-9β,10α-progesterone (generic name, 'Dydrogesterone') (I) and 9β,10α-progesterone (II), were found to exert a markedly progestational activity when administered



III



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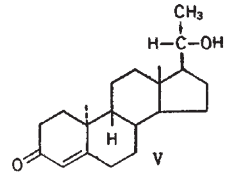

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