Gas Chromatographic Analysis of Ecdysone

Moulting and metamorphosis in insects is controlled by steroid hormones of the ecdysones group. One of these, α-ecdysone, is the principal moulting hormone in insects[1]. Following the isolation, elucidation of its structure[2] and synthesis, this hormone is now commercially available.

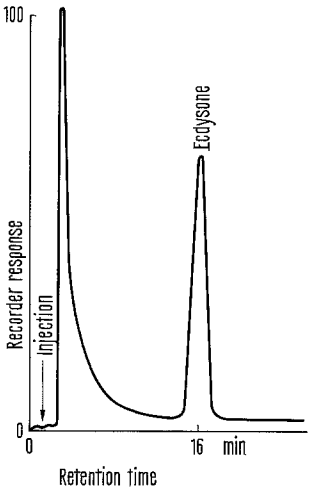
The level of ecdysone in insects has until now been determined by bioassays with *Calliphora erythrocephala*[3], *Musca domestica*[4] and other insects. Although, these assays are very sensitive, their evaluation is based on the percentage of puparium formation in treated animals. An attempt was made to develop a method which would permit a quantitative analysis of α-ecdysone. For this purpose gas-liquid chromatography was used.

α-ecdysone was analyzed as a derivative of *Bis* (Trimethylsilyl) Acetamide (Applied Sci., Pa. USA). Up to 1 mg of α-ecdysone (Hoffmann-La Roche, Basel[5]) was converted into the above derivative using 0.5 ml *Bis* (Trymethylsilyl) Acetamide with 0.1 ml pyridine. The solution was heated to 80 °C for 1 min and directly injected with the reagent into the gas chromatograph.

The sample was analyzed on a Packard gas chromatograph equipped with a flame ionization detector, using an all glass column (3′ × 1/8″) packed with 3% SE-30 on gas-Chrom Q (Applied Sci.). Operating temperatures for the inlet, column and detector were 290, 250, and 280 °C, respectively. Nitrogen was used as a carrier gas at a flow rate of 100 ml/min.

The ecdysone peak appeared 16 min after injection, as shown in the Figure. All other steroids including cholesterol and phytosterols came out with the peak of the solvent and did not interfere with the analysis. Using this procedure 50 ng of α-ecdysone could be detected.

Further investigations are in progress to determine the concentration of this hormone in insects.



Gas chromatogram of 100 ng α-ecdysone.

Résumé. Nous avons développé une méthode d'analyse micro-quantitative de l'ecdysone (hormone de la mue des insectes) par chromatographie gazeuse. Elle permettera l'analyse chimique de l'ecdysone, qui a été dosée jusqu'à présent par des méthodes biologiques.

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[1] P. Karlson, Annls Sci. nat., Zool. *18*, 125 (1956).
[2] P. Karlson, H. Hoffmeister, H. Hummel, P. Hocks and G. Spittler, Chem. Ber. *98*, 2394 (1965).
[3] A. Butenandt and P. Karlson, Z. Naturforsch. *9b*, 389 (1954).
[4] D. Adelung and P. Karlson, J. Insect Physiol. *15*, 1301 (1969).
[5] We are indebted to Mssrs Hoffmam-La Roche, Basel, for kindly supplying the α-ecdysone.
[6] Supported by a grant from the Authority for Research and Development of the Hebrew University and by the Israel Academy of Sciences.

An Apparatus for Investigation of Heterogeneous Reactions in a Flow System

Devices of different types have been reported for the study of heterogeneous reactions; a 'one shot' micro reactor[1], a one batch reaction device[2], a pulse reactor[3] and a flow system reactor[4]. Of these, assemblies of the last type are especially useful in the study of catalytic reactions[5].

The apparatus described here is a simple and versatile device for the study of reactions in a continuous flow system at temperatures up to 700 °C. It can be used for liquids, gases or a mixture of the two in the presence of a solid catalyst or an inert support, with or without dilution with suitable carrier. The reactants may be fed separately into the reaction chamber at various flow rates so that mixing takes place only in the reaction chamber. The reaction time can be predetermined and the liquid hourly space velocity (LHSV)[6] can be measured.

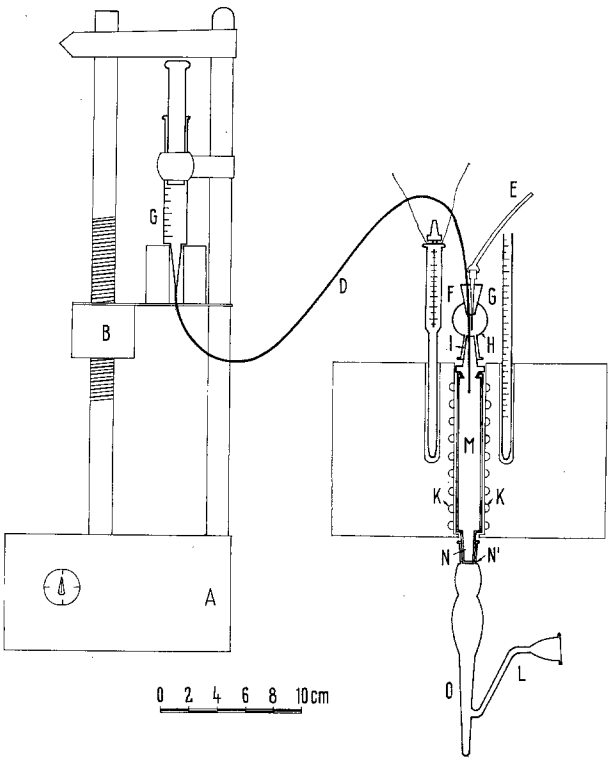
Method. The apparatus is composed of 3 main parts (Figure). 1. A feeding device, 2. a reaction chamber and 3. a collector.

1. The feeding device is composed of a Bird Kymograph (A) (Phipps and Bird Inc., Richmond, Virginia, USA, Cat. No. 70-060), in which the shaft is replaced by a threaded one (B) connected to a platform which can thus be raised or lowered. A syringe (c) is located between the platform and the upper shaft holder so that the raising of the platform depresses the plunger and the sample in the syringe is introduced into the reaction chamber (M) at a predetermined velocity through the capillary (D) and hypodermic needle (F). If a carrier gas is used, it is introduced through a second capillary whose terminus (G) is placed about 20 mm above the outlet of the sample needle in order to prevent the con-

[1] R. J. Kokes, H. Tobin and P. H. Emmett, J. Am. chem. Soc. *77*, 5860 (1955).
[2] H. Pines and J. Ryer, J. Am. chem. Soc. *77*, 4370 (1955).
[3] P. Steingaszner and H. Pines, J. Catalysis *5*, 356 (1966).
[4] H. Pines and S. M. Csicsery, J. Catalysis *1*, 313 (1962).
[5] G. M. Schwab, in *Advances in Catalysis and Related Subjects* (Academic Press Inc., New York, 1950), vol. II, p. 251.
[6] V. Haensel, Ind. Engng Chem. *57*, 18 (1965).

densation or evaporation of the sample in the upper part of the reaction chamber. The syringe is held in position with outlet downward, and a bubble of air or any other gas above the sample in the syringe insures that the whole sample is transferred to the reaction chamber. In a similar manner a number of reactants may be introduced into the chamber simultaneously in varying ratios.

2. The reaction chamber is a cylindrical brass block (M) (length 115 mm; O.D. 20 mm; wall 2 mm) which has 2 standard ground male joints (10/19 at the upper end (I) and 14/24 at the lower end (N)). The catalyst is inserted or removed from the reaction chamber by removing the upper end (P) of the block, which is fitted in place with a ground joint. A standard ground female joint capped with a rubber septum (H) is connected to the inlet of the reaction chamber. The injection needles (F, G) pass through the rubber septum into the cylinder (M). The reaction chamber is located in a cylinderical heating block (length 120 mm; I.D. 20.5 mm) which is wound with 2 Cr-Ni wires (K). One of these (100 W at 220 V; total resistance 400 Ω), connected to a variable resistance, is used for coarse adjustment of the temperature (± 2 °C), while the other (170 W at 220 V, total resistance 270 Ω), connected to a variable contact-thermometer (W) (Jumo Inc., Fulda, Germany, Cat. No. MS-DBP.12.64) serves for fine control (± 0.25 °C at 500 °C). A variable-contact thermometer and an ordinary thermometer (U) are positioned in 2 sockets located at equal distances of 10 mm from the reaction chamber and at a depth of 60 mm. The heating block is insulated with a light magnesium oxide housing (200 mm × 200 mm × 120 mm).



Scale diagram of the apparatus. (A) Kymograph; (B) threaded shaft with platform; (C) syringe; (D) sample capillary; (E) carrier gas feed; (F) hypodermic needle; (G) capillary for the carrier gas; (H) female joint with rubber septum; (I) upper male joint; (K) heating elements; (L) receiver side arm; (M) reaction chamber; (N) lower male joint; (N') receiver female joint; (O) conical receiver.

3. The collecting device is connected to the lower joint of the reaction chamber. It is a conical receiver (O) with an S shaped side arm (L) (total volume 15 ml) and a bulb in its upper part. The latter was found to prevent escape of products through the side arm. The lower part of the receiver is externally cooled, and a protecting tube is connected to the side arm. Gases not trapped in the reciver may be collected by connecting the side arm to a gas collector (a rubber bulb or a dilatometer).

An example of a typical reaction that has been studied with this apparatus is the base catalyzed isomerization and concurrent aromatization of menthadienes. The results of repeated experiments in which 50 µl of liquid reactant was injected into the reaction chamber at a rate of 31.2 µl per min and carried in a stream of oxygen-free nitrogen (flow rate 1.3 ml per min) through 28 g of CaO (15 mesh) catalyst, are shown in Tables I, II and III. Material recovery (in acetone-dry ice cooled trap) was above 99% in all cases.

As can be seen, the reproducibility of the results is very good, being much better than those reported for other flow system instruments[4]. In most cases the standard deviation is in the range of ± 0–4%. Only in few cases, primarily those in which the product in question appears in very small amounts, is it in the range of ± 5–10%. The greater variability is the result of uncer-

Table I. Isomerization and aromatization of terpinolene over a CaO catalyst at 380 °C

% Products[a] Products	Run No. 1	Run No. 2	Run No. 3	Run No. 4	Run No. 5	Average and S.D.
α-Terpinene	19.0	18.5	20.0	19.0	20.0	19.3 ± 0.5
2,4(8)-Menthadiene	12.0	12.0	13.0	12.0	13.0	12.4 ± 0.5
Limonene	0.5	0.5	0.5	0.5	0.5	0.5 ± 0.0
3,8(9)-Menthadiene	5.0	5.0	5.0	5.0	5.0	5.0 ± 0.0
Terpinolene	2.0	2.0	2.0	2.0	2.0	2.0 ± 0.0
γ-Terpinene	5.0	6.0	5.0	6.0	5.0	5.4 ± 0.5
p-Cymene	47.0	48.0	47.0	48.0	47.0	47.4 ± 0.5
p-Isopropenyltoluene	0.5	0.5	0.5	0.5	0.5	0.5 ± 0.0
Pyrolitic Products	8.5	7.5	6.5	7.0	6.5	7.2 ± 0.7

[a] Determined from the area ratio of the products as obtained on a v.p. chromatogram (5% tetrachlorophthalate and 10% Carbowax-20M on Chromasorb-W columns).

Table II. Isomerization and aromatization of terpinolene over CaO catalyst at 300 °C

% Products[a] Products	Run No. 1	Run No. 2	Run No. 3	Run No. 4	Average and S.D.
α-Terpenene	24.4	24.0	24.0	24.0	24.1 ± 0.3
2,4(8)-Menthadiene	30.0	30.0	30.0	30.0	30.0 ± 0.0
Limonene	1.0	1.0	1.0	1.0	1.0 ± 0.0
3,8(9)-Menthadiene	9.0	9.0	9.0	9.0	9.0 ± 0.0
Terpinolene	1.0	1.0	1.0	1.0	1.0 ± 0.0
p-Isopropenyltoluene	2.5	2.0	2.5	2.0	2.3 ± 0.2
γ-Terpinene	9.0	9.0	9.0	9.0	9.0 ± 0.0
p-Cymene	16.0	17.0	16.0	17.0	16.5 ± 0.5
Benzene + Toluene	2.0	2.0	2.0	2.0	2.0 ± 0.0
m-Xylene	1.0	1.0	1.0	1.0	1.0 ± 0.0
Pyrolitic products	4.0	4.0	4.0	4.0	4.0 ± 0.0

[a] See footnote Table I.

Table III. Isomerization and aromatization of limonene over CaO catalyst at 475 °C

% Products[a] Products	Run No. 1	Run No. 2	Run No. 3	Average and S.D.
Limonene	45.0	44.5	44.5	44.7 ± 0.2
α-Terpinene	5.5	6.0	6.0	5.8 ± 0.3
Terpinolene	3.5	3.5	3.0	3.3 ± 0.2
2,4(8)-Menthadiene	5.0	5.0	5.0	5.0 ± 0.0
γ-Terpinene	2.5	2.5	2.5	2.5 ± 0.0
p-Cymene	23.0	23.5	23.0	23.2 ± 0.2
p-Isopropenyltoluene	3.0	3.0	3.0	3.0 ± 0.0
Benzene	2.0	2.0	2.0	2.0 ± 0.0
Toluene	1.0	1.0	1.0	1.0 ± 0.0
m-Xylene	1.5	1.5	1.5	1.5 ± 0.2
Pyrolytic products	7.0	6.5	7.0	6.8 ± 0.2

[a] See footnote Table I.

tainty in measurement of small areas in the v.p. chromatograms[7].

Zusammenfassung. Es wird eine Apparatur zum Studium heterogener und pyrolytischer Reaktionen in Fliess-Systemen beschrieben, die für flüssige und gasförmige Ausgangsstoffe, Reaktionsprodukte sowie für deren Mischungen geeignet ist.

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[7] Acknowledgment. This work was partially supported by a grant from Bar-Ilan University Research Committee.

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Rapid Microestimation of Proteins by Membrane Chromatography on PVC Ultrafilters

In a previous communication we described a simple and rapid method for the quantitative estimation of proteins by radial chromatography on nitrocellulose membrane filters[1,2]. However, that technique had the following limitations: Low-molecular proteins (mol wt. below about 100,000) could be adsorbed to nitrocellulose only at acid pH values, e.g. 3.7, and for proteins having mol wt. below about 40,000 that estimation usually failed because of their insufficient adsorption on the carrier[3].

The limitations mentioned above seem to be surmountable in a simple way by using polyvinylchloride (PVC) membrane filters instead of nitrocellulose, since our preliminary experiments with PVC membranes[4] revealed that they adsorb proteins quite feasibly in a range of mol wt. about 12,000–400,000 and pH 3.7–9.06. The present paper brings more data confirming the above findings, reports new details of membrane chromatography on PVC and presents a series of calibration curves of various proteins.

Material and methods. PVC membranes Sartorius (Göttingen, Germany) SM 12801 were used in strips 3–4 × 10–20 mm, wetted in 40% aqueous ethanol and washed thoroughly by a 0.1 *M* phosphate buffer pH 7.2 which had been diluted with 0.9% NaCl 1:1. The same buffer was used for one-dimensional ascending chromatography as described in reference [4]. Bovine serum albumin, human γ-globulin and fibrinogen, ovalbumin, horse myoglobin[5] and bovine ribonuclease (Reanal) diluted in the developing buffer to 0.2% concentration were applied by means of a thin capillary calibrated by 1 μl[1,2] stepwise to the surface of a plexi glass slide in successive portions of about 0.3 μl. One calibrated capillary was used in all experiments. The droplets were quantitatively soaked into the starting edge of the wet membrane which stuck spontaneously to a supporting glass at the other end (Figure 1) and was then gently pressed with the fingers between that glass slide and a dry filter paper wick Whatman No. 1. The strips were then immediately developed for about 1–3 min until the flow marker, e.g. 10% potassium bichromate, reached the upper end. After staining the membranes with 0.5% amidoblack 10B in 5% trichloroacetic acid and destaining in water (Figure 2), the area of the protein layer was measured by means of

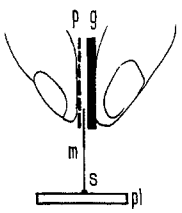

Fig. 1. Application of the sample to the PVC strip. p, dry filter paper wick; g, supporting glass plate; m, PVC membrane filter; (p, g and m are gently pressed together by fingers); s, sample; pl, plexi glass.

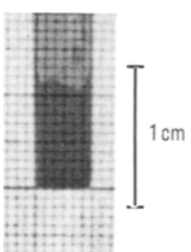

Fig. 2. Stained protein film developed chromatographically on a PVC membrane. 2 μl of 0.2% bovine serum albumin was developed in phosphate buffer pH 7.2 on PVC membrane SM 12801 for about 2 min. Stained with amido black 10 B. The transparent scale is placed upon the strip.

[1] T. I. Přistoupil, Nature *212*, 75 (1966).
[2] T. I. Přistoupil, M. Kramlová and J. Štěrbíková, J. Chromat. *34*, 370 (1968).
[3] T. I. Přistoupil, M. Kramlová and J. Štěrbíková, J. Chromat. *42*, 367 (1969).
[4] T. I. Přistoupil, J. Chromat., *49*, *550* (1970).
[5] Prepared in our Institute.