A CONTRIBUTION TO THE DETERMINATION OF 2-[2-(CHLOROPHENYL)-2-PHENYLACETYL]-1,3-INDANEDIONE (CHLOROPHACINONE)

By

S. BALOGH,* K. ERŐSS-KISS, S. TÖRÖK* and I. ZÁMBÓ***

Department of General and Analytical Chemistry, Technical University Budapest

Received September 8, 1981

Presented by Prof. Dr. E PUNGOR

In biological plant protection the most widespread rodenticides are cumarin and indanedion derivatives, based on their anticoagulant effect [1]. The purpose of this paper is to present analytical methods suited to determine the active agent content in REDENTIN products manufactured and marketed by REANAL Chemicals, Budapest.

The active agent in REDENTIN products is the 2-acyl-1,3-indanedione derivative chlorophacinone (2-[2-(4-chlorophenyl)-2-phenylacetyl]-1,3-indanedione). REDENTIN is commercialized in the form of REDENTIN-0, concentrates of the active agent in paraffin oil used in redenticide formulations (REDENTIN-0-0,25,-0-0,5,0-1 and -0-2, containing 0.25, 0.5, 1 and 2%, resp., of the active agent) and in the form of REDENTIN 75, a rodenticide in which the active agent is applied on crushed maize corns.

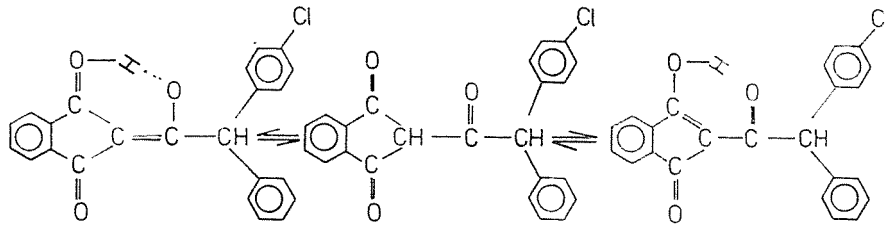
It is known that the active agent content of rodenticides based on 2-acyl-1,3-indanedione derivatives can be determined — after previous separation by column chromatography [2] or thin-layer chromatography [3, 4, 5] — by spectrophotometry [6], spectrofluorimetry [7] and polarography [8].

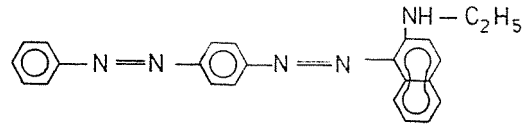
We developed a novel infrared spectrophotometric technique to determine the active agent content of the oily concentrates REDENTIN-0 without requiring previous separation.

* REANAL Chemicals, Budapest

*** Research Institute of Medicinal Plants, Budakalász

IR spectrophotometric determination of chlorophacinone (I) in paraffin oil solutions in the presence of N-ethyl-(4(phenylazophenylazo)-2-naphthylamine disazo dyes (II)





II

In order to find the analytical sites suited for the determination of the active agent content, we first studied the IR spectra of the components present in the oily solution. We found that the bands marked with × in Fig. 1 are suitable for analysis, since in these wavelength ranges both the disazo dye (II) applied in a concentration of 0.2% as warning dye and paraffin oil have only a grey-body absorption.

Based on these preliminary studies, we selected the C=O band appearing at the highest frequency $\nu = 1705\ cm^{-1}$ for determining the active agent content. The active agent is present in the enol-A or enol-B form.

In the spectra taken of the calibration series we determined the absorbance of the band appearing at 1705 cm^{-1} by the base line correction technique.

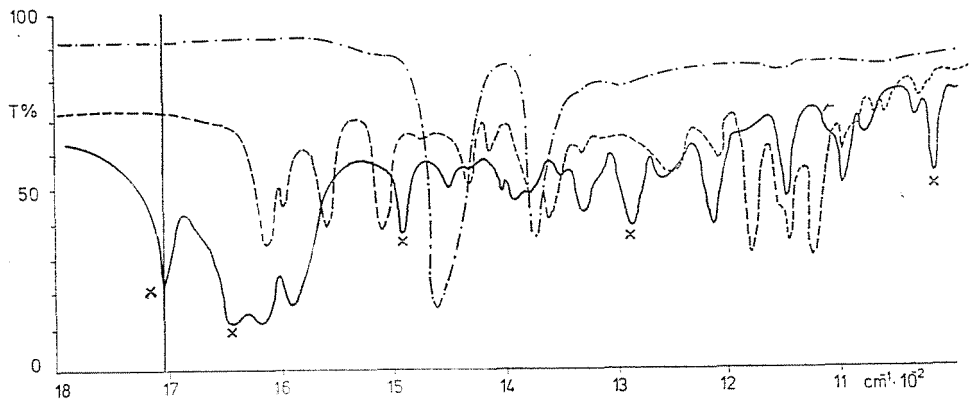


Fig. 1. Selection of the analytical site in IR determination of chlorophacinone
———— Chlorophacinone (I)
— — — — — Sudan Red 7B (II)
—.—.—.—.— Paraffin oil

The function absorbance *vs.* concentration obtained is presented in the calibration line Fig. 2.

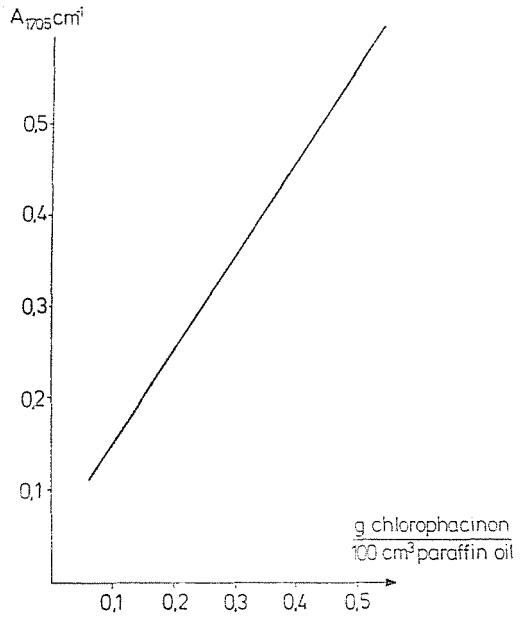


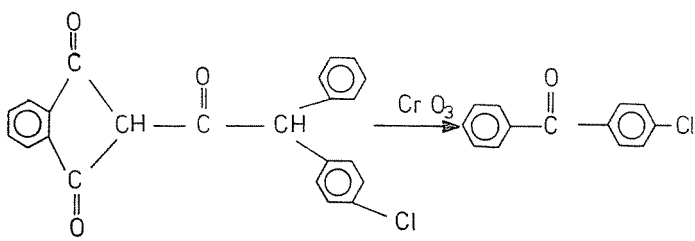
Fig. 2. Calibration curve for the determination of chlorophacinone at the wavelength 1705 cm^{-1}

Determination of chlorophacinone by gas chromatography

The active agent can also be determined by gas chromatography both in the paraffin oil solutions REDENTIN-0 and in the rodenticide REDENTIN 75.

The concentration of the active agent was measured with close to identical accurracies with both IR and GC techniques in paraffin oil concentrates. In contrast, gas chromatography is better suited for the analysis of REDENTIN-75, since maize oil will be extracted together with the active agent in the preliminary extraction needed, and its IR absorption bands will partly overlap with the bands utilized in the determination.

The gas chromatographic process first reported by BULLARD and coworkers [9] consists in an oxidation with chromic acid, providing, among other reaction products, 4-chlorobenzophenone:



This compound can be determined quantitatively by gas chromatography; using an electron capture detector and conditions listed in the experimental part of this paper, relatively low concentrations of the active agent will be determined with satisfactory accuracy.

In the present study, our aim was to find optimum conditions of the oxidation with chromic acid: amount of oxidizing agent, reaction time and reaction temperature.

To determine the optimum amount of the oxidizing agent, amounts of the active agent varying between 20 and 400 μg were brought into reaction with a 2.5% solution of CrO_3 in acetic acid.

GC analyses demonstrated that within this concentration range, 8. . .10 cm^3 oxidizing agent was sufficient, since no further increase of 4-chlorobenzophenone resulted from further addition.

The rate of reaction was studied at boiling water bath temperature and 120 °C sand bath temperature. The results obtained in the sand bath were superior regarding reproducibility, presumably because at this temperature the reaction mixture is boiling and hence ensures constant temperature. Also, the reaction was found to be more complete.

As to reaction time, we stated that the maximum amount of 4-chlorobenzophenone is obtained from the pure active agent after boiling for 20. . .30 minutes at 120 °C. Further boiling results in a decrease of 4-chlorobenzophenone, owing to its oxidative decomposition. It is therefore eminently important to keep reaction time of 30 minutes under strict control.

At the above experimental conditions, around 90% of the active agent present in amounts of 20. . .400 μg will be oxidized to 4-chlorobenzophenone.

The gas chromatographic integrator response changes linearly with 4-chlorobenzophenone concentrations corresponding to 2. . .16 μg amounts of active agent when an electron capture detector is utilized, and to 20. . .50 μg amount of active agent when a flame ionization detector is utilized. For quantitative determination, 4-chlorobenzophenone obtained by the oxidation of a known amount of the pure active agent was used as internal standard.

Experimental

1. Infrared spectrophotometric analyses

A Zeiss UR 10 spectrophotometer was used in these studies. To select the analytical site, we applied the potassium bromide tablet technique (2 mg sample per 1 g KBr) to record the spectra of the individual components.

Detailed description of the procedure. A stock solution was prepared by by dissolving 0.500 g of the active agent (I) and 0.400 g of the dye (II) in 100.0 cm^3 paraffin oil.

The calibration series was prepared by diluting the stock solution by a factor of 1.5; 2; 3 and 4 with paraffin oil. Simultaneously a refernce calibration series containing no active agent was prepared.

The calibrating solutions and the corresponding reference solutions were introduced into cuvettes fitted with 1.00 mm sodium chloride windows. The spectrograms were recorded at a rate of 50 cm^{-1}/min in the interval of the analytical sites, placing the calibrating solution in the path of the measuring ray and the reference solution in the path of the reference ray. After plotting the calibration curve, the absorbance of the oily concentrate is measured at 1705 cm^{-1}.

The accuracy of the determination is $\pm 5\%$ (relative).

2. Gas chromatographic analyses

A JEOL JGC 1100 instrument and a ^{63}Ni electron capture detector were used in these analyses. The glass column was 1.5 m in length and 3 mm in inner diameter. The packing applied was Gas Chrom Q (80—100 mesh) containing 4% SE-30 + 6% OV-210 distributing liquid.

Temperature of the injection device and the detector: 240 °C.

Temperature of the column space: 180 °C.

Injected amount: 1. . .3 μl.

Carrier gas: high-purity nitrogen at a pressure of 147.1 kPa.

At these conditions the retention time of 4-chlorobenzophenone was 5 minutes.

Detailed description of the procedure

Chemicals:

Chromic acid solution (2.5 g CrO_3 are dissolved in 1.5 cm^3 water and made up to 100 cm^3 with glacial acetic acid)

n-Hexane (analytical grade)

Acetone (analytical grade)

Sodium sulfate, anhydrous (analytical grade)

a) Paraffin oily concentrates. 2.00 cm^3 of the concentrate are pipetted into a 100.0 cm^3 volumetric flask and made up to the mark with acetone. For oxidation, 5.00. . .10.000 cm^3 are pipetted into a 50 cm^3 ground-neck, round-bottom flask and evaporated to dryness in a rotating vacuum evaporator.

b) 10.0 g of the crushed maize rodenticide are extracted in a Soxhlet apparatus with 70 cm^3 acetone for 2 hours. The solution is then washed with acetone into a 100.0 cm^3 volumetric flask and made up to the mark with acetone. Depending on the concentration of the active agent, 10.00. . .50.00 cm^3 of this solution are pipetted into a ground-neck, round-bottom flask and evaporated to dryness in a rotating vacuum evaporator.

The residue obtained as described in a) and b) is then refluxed for 30 minutes with 10 cm^3 chromic acid solution on a sand bath at 120 °C. After cooling, the contents are transferred into a 100 cm^3 separating funnel. The flask is washed with 2×5 cm^3 water, the water is added to the liquid in the funnel.

4-Chlorobenzophenone to be subjected to chromatography is then extracted by shaking with 18 cm^3 *n*-hexane. The aqueous-acid phase is discarded, the hexane phase is washed acid-free with 3×20 cm^3 distilled water and filtrated through anhydrous sodium sulfate. The funnel and the sodium sulfate are washed with 3 cm^3 *n*-hexane each. All hexane solutions are collected in a 25.00 cm^3 volumetric flask and made up to the mark with *n*-hexane.

For calibration, pure 2-[2-(4-chlorophenyl)-2-phenylacetyl]-1,3-indanedione is oxidized in a similar manner. An aliquot part (50. . .500 μg) of an acetonic solution of 5.00. . .10.00 mg of the active agent is evaporated to dryness and oxidized as described above, and 4-chlorobenzophenone obtained in the reaction is subjected to gas chromatography. The calibration curve is plotted on the basis of either the area under the chromatographic peak, or the integrator value, *versus* the concentration of the pure active agent applied.

The accuracy of the determination is $\pm 5\%$ for measurements of 0.25% active agent concentrations; for 0.005. . .0.075% concentrations, it is $\pm 10\%$.

Note:

If the specimens to be analyzed contain more than 0.25% of the active agent, the dilutions should be adjusted in a manner that gas chromatographic signals and absorbance values measured at 1705 cm^{-1} should lie on the linear sections of the corresponding calibration curves.

Summary

We developed a direct IR spectrophotometric procedure to determine the active agent 2-[2-(4-chlorophenyl)-2-phenylacetyl]-1,3-indanedione in REDENTIN O, its concentrates in paraffin oil. The band suitable for analytical purposes is sited at 1705 cm^{-1}.

For determining this active agent in REDENTIN-75, a rodenticide in which it is applied on crushed maize, we used the gas chromatographic method reported by BULLARD and coworkers [9] after previous extraction with acetone. Our studies resulted in finding the optimum conditions for the oxidation of the active agent with chromic acid, and the concentration limits between which the active agent can be determined with satisfactory accuracy using an electron capture detector and a flame ionization detector, respectively.

References

1. WEGLER, R.: Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel. Bd. I, pp. 614—644. Berlin, Springer, 1970.
2. MARTIN, H.: Pasticide Manual. P. 114. British Crop. Protection Council, 1972.
3. RÜSSEL, H. A.: Z. für Anal. Chem. **250**, 125 (1970).
4. DAENENS, P., VAN BOUVEN, M.: J. Chromatogr. **79**, 217 (1973).

5. MALLET, V.—SURETTE, D.—BRUN, G. L.:J. Chromatogr. **79**, 217 (1973).
6. CASWELL, R. L.: J. Assoc. Offic. Agr. Chem. **42**, 104 (1959).
7. HOLLIFIELD, H. C.—WINEFORDNER, J. D.: Talanta. **14**, 103 (1967).
8. DANEK, A.—KWIEK, J.: Dissertationes Pharmaceuticae Acad. Med. Cracow. **16**, 359 (1964).
9. BULLARD, R. W.—HOLGUIN, G.—PETERSON, J. E.: J. Agric. Food Chem. **23**, 72 (1975).

Sándor BALOGH — REANAL Chemicals H-1441 Budapest 70. Pf. 54
Dr. Klára ERŐSS-KISS — H-1521 Budapest
Sándor TÖRÖK — REANAL Chemicals H-1441 Budapest
István ZÁMBÓ — Research Institute of Medicinal Plants, Budakalász