Preparation of 2,3-Dinitrilo-1,4-dithia-9,10-anthraquinone Labelled with ^{14}C or ^{35}S or $^{14}C+^{35}S$

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SUMMARY

A method was developed for the preparation of 2,3-dinitrilo--1,4-dithia-9,10-anthraquinone labelled with ^{14}C or ^{35}S or $^{14}C+^{35}S$ on a milligram scale with the use of the semiconductographic detection. The exchange of the ^{35}S radionuclide in various stages of the preparation was examined in detail. By the present method, the following activities were obtained: 88 Ci of ^{14}C/mol or 44 Ci of ^{35}S/mol or 1.46 Ci of ^{14}C + 75 Ci of ^{35}S/mol.

INTRODUCTION

In the chemistry and biochemistry of fungicides, considerable attention has been paid to substances possessing the grouping[1] -S/CN/: C/CN/S-. Several derivatives of this type exhibit remarkable pesticidal and fungicidal properties.[2-7] An excellent fungicidal effect has been observed with 2,3-dinitrilo-1,4-dithia-9,10-anthraquinone[8-10] indexed by Chemical Abstracts as 5,10-dihydro-5,10-dioxonaphtho[2,3-b]-1,4-dithiin-2,3-dicarbonitrile under the code number [3347-22-6], for the sake of brevity, the trivial name dithianon is used in the further text. The manufacture and some application of dithianon have been object of patents.[1,11-15] In addition to the fungicidal activity, dithianon also exhibits acaricidal, insecticidal, nematocidal, herbicidal, and repellent effects.[16,17]

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In agriculture, dithianon is for example used against Venturia inaequalis,[18,19] Alternaria porri,[20] Podosphaera leucotrichia,[2,19] Tranzschelia discolor,[3] Xanthomonas oryzae,[4] Piricularia oryzae[4] and numerous other fungal or bacterial diseases of plants. In view of the wide spectrum of effects, low toxicity, and high biological activity, dithianon is also of interest in other fields, particularly in molecular biology and biochemistry.[21-22]

The aim of the present work was to develop a method for the preparation of dithianon labelled with ^{14}C, ^{35}S or $^{14}C+^{35}S$ on a milligram scale. In the preparation and assays on the radiochemical stability of the dithianon thus labelled, use was successfully made of semiconductography,[23] the single radiometric method for a non-destructive resolution of the ^{14}C- and ^{35}S- activity in the same sample.[24]

EXPERIMENTAL

Reagents

Reagents /Lachema, Brno, Czechoslovakia/ were of the Analytical Grade purity: 1-naphthol, sodium chlorate, concentrated sulfuric acid, glacial acetic acid. Potassium cyanide was recrystallised from boiling ethanol. Dimethylformamide was purified according to ref.[25] The labelling was performed with potassium cyanide-^{14}C 0.73 Ci/mol and 44 Ci/mol, Institute for Research, Production, and Application of Radioisotopes, Prague, Czechoslovakia and the ^{35}S radionuclide in the form of elemental sulphur 4 Ci/g, Izotop, Soviet Union .

Methods and Instruments

Ultraviolet spectrometry was performed by means of Unicam SP 800 /PYE UNICAM Ltd., England/, infrared spectrometry in VEB Carl Zeiss equipment /Jena, GDR, Model UR 20/.

Thin-layer chromatography /TLC/ was carried out on ready--for-use Silufol UV-254 /Kavalier Glassworks, Votice, Czechoslovakia/ silica gel sheets containing a fluorescent indicator. Column chromatography was performed on the macroporous silica gel /produced by Service Laboratories of this Institute/, previously deactivated partly by addition of water /16%/.

All reaction steps in the microscale preparation were advantageously performed in a closed all-glass apparatus[26] adapted for a low-temperature vacuum sublimation under cooling with liquid nitrogen.

Activity of the particular discrete samples was measured in a Tri-Carb scintillation spectrometer /Packard Instrum. Company, USA, 3375 Model/. Non-destructive detection in a developed chromatographic layer were carried out on a semiconductographic device.[23,24,27] The chromatogram was fixed to a moving table, whose motion in an orthogonal coordinate system was controlled by an attached computer. The step-lenght in both directions was adjusted to 1 millimeter, the quantitative evaluation was made possible by the geometrical arrangement of the detector, the diaphragm /aperture, 1x1 mm/, and the chromatographic layer.[27] Voltage impulses from the detector were amplified and then recorded in parallel in 18-170 keV and 156-170 keV channels. Counting rates per the time interval used in particular channels were recorded for each site both numerically on a printing device and by means of dots differring in colour according to orders of magnitude. In the case of samples labelled simultaneously with ^{14}C and ^{35}S, the values of the particular radionuclides were calculated from values observed in both channels analogously to other radiometric methods, e.g., liquid scintillation spectrometers.[28]

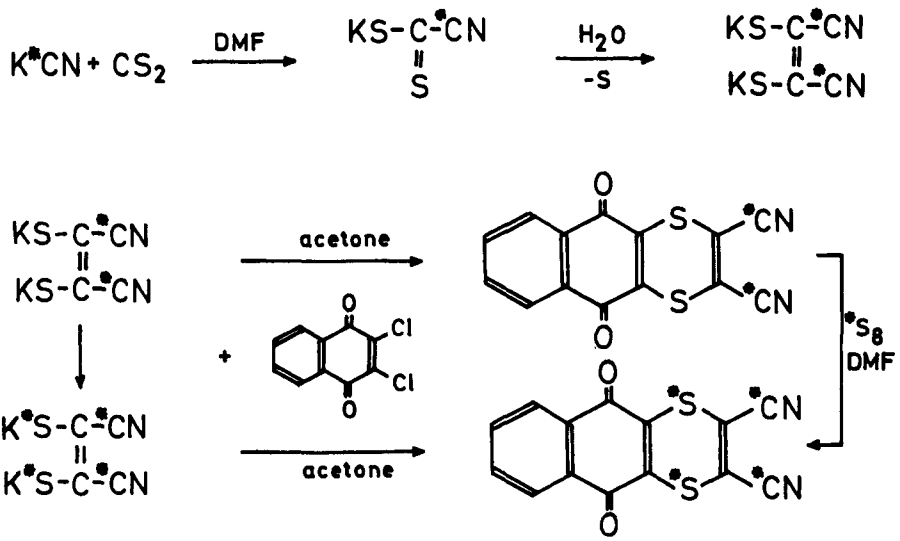
Preparation of Labelled Compounds

The synthetic procedure[11-13] was modified for the preparation of labelled compounds on a microscale. The experiments can be illustrated by the Scheme on the next page.

Preparation of Dithianon-^{14}C

Potassium cyanide-^{14}C /4.1 mg/ and dimethylformamide /0.5 ml/ were introduced into a test-tube equipped with a magnetic stirrer. The mixture was briefly agitated, frozen down, treated with carbon disulfide /10 µl/, and then vigorously stirred in the tightly stoppered vessel for 30 min. The mixture gradually became brown-yellow owing to the formation of potassium cyanodithioformate /NC-CS-SK/. The dimethylformamide was evaporated, the residue triturated with ether /0.5 ml/, and the ether evaporated. The residual crystals were dissolved in water /0.5 ml/, the aqueous solution /turbid due to the deposit of sulphur/ was kept at room temperature for 24 hours, frozen down by means of liquid nitrogen, and the water removed by freeze-drying under diminished pressure. The resulting 1,2-dicyanoethene--dithiol potassium salt was dissolved in acetone /0.5 ml/ and a solution of 2,3-dichloro-1,4-naphthoquinone /4 mg, 0.09 mmol, for the preparation see ref.[29]/ in acetone /1 ml/ was added. The mixture was briefly agitated and the acetone removed by sublimation in vacuo. The residue was dissolved in benzene /0.2 ml / and the solution subjected to column chromatography.

Scheme



Preparation of Dithianon-^{35}S by Exchange of-^{35}S into 1,2-Dicyanoethenedithiol Potassium Salt

Elemental sulphur-^{35}S /1.25 mg/ in dimethylformamide /1.0/ was added into a solution of 1,2-dicyanoethenedithiol potassium salt /8.0 mg, 7 μmol, prepared according to ref.[30,31]/ in dimethylformamide /0.5 ml/, the whole transferred into an ampoule, and frozen down, the ampoule was evacuated, sealed, and heated for 30 min. in a test-tube equipped with a condenser and containing refluxing dimethylformamide. The content of the ampoule was then transferred into a test-tube equipped with magnetic stirrer and frozen down. The dimethylformamide was evaporated under diminished pressure and the residue treated with 2,3-dichloro-1,4-naphthoquinone /8.5 mg, 40 μmol/ in acetone /2 ml/. The mixture was briefly stirred, the acetone evaporated, the residue dissolved in benzene /1 ml/, and the solution subjected to two column chromatographies.

Preparation of Dithianon-^{35}S by a Direct Exchange with ^{35}S

Chromatographically pure dithianon /5 mg, 16.6 μmol/ was dissolved in dimethylformamide /1 ml/, the solution treated with elemental sulphur-^{35}S /1 mg/ in dimethylformamide /2 ml/, and the whole refluxed for 5 min. in a test-tube equipped with a reflux condenser. The reaction mixture was then cooled down, the dimethylformamide removed by sublimation under diminished pressure, the residue dissolved in benzene /0.5 ml/, and the solution subjected to column chromatography.

Preparation of Dithianon-^{14}C, ^{35}S

A mixture of the above prepared 1,2 dicyanoethenedithiol-$^{14}C_2$ potassium salt /8.0 mg, 37 μmol/, elemental sulphur ^{35}S /1.25 mg/, and dimethylformamide /1.0 ml/ was transferred into an ampoule which was frozen down, evacuated, sealed, and heated at 155°C for 30 min. in a dimethylformamide bath. The content of the ampoule was then frozen down, the dimethylformamide evaporated under diminished

pressure, the residue dissolved in acetone /0.5 ml/, and the solution treated with 2,3-dichloro-1,4-naphthoquinone /9.0 mg, 42.5 μmol/ in acetone /3.0 ml/.

The acetone was removed by freeze drying, the residue dissolved in benzene /0.5 ml/, and the solution subjected to column chromatography.

RESULTS AND DISCUSSION

The exchange of ^{35}S was examined in all reaction stages under various conditions such as in the presence of air, in the absence of air and under diminshed pressure, and in the medium of pyridine or dimethylformamide.

As indicated by these experiments, the following exchanges can be used on a preparative scale: exchange of ^{35}S in 1,2-dicyanoethenedithiol potassium salt, in dimethylformamide as solvent, under diminished pressure /Table I/ and exchange of ^{35}S in dithianon, in dimethylformamide, in the presence of air /Table II/ despite the discouraging reports on the difficult exchange of this heterocyclic sulphur in the literature.[32]

The identity of the dithianon ^{35}S thus prepared was therefore examined with a special attention. The specimen was purified by chromatography and compared with an in active sample prepared according to ref.[11-13] with the above mentioned modifications

Samples of dithianon and dithianon ^{35}S exhibited identical data of the melting point /225°C/, UV spectrum / max 354 nm in ethanol/, IR spectrum /Fig.1/ and R_F /0.63/ on a chromatographic thin-layer in benzene /twice developed/.

TABLE I

Exchange of ^{35}S into 1,2-Dicyanoethenedithion Potassium salt in Dimethylformamide as Solvent

Time min.	Exchange degree,% $^{32}S \rightleftharpoons ^{35}S$	Yield,% Radiochem.	Yield,% Chemical	Radiochemical purity,%
10	89.5	18.6	21.4	97.2
30	99.2	17.3	17.4	96.2
60	91.6	15.6	17.1	85.7
120	35.6	5.3	7.5	91.0

TABLE II

Exchange of ^{35}S into Dithianon

Time min.	Exchange degree,% $^{32}S \rightleftharpoons ^{35}S$	Yield,% Radiochem.	Yield,% Chemical	Radiochemical purity,%
0	4.7	3.5	75.4	72.5
5	60.0	36.4	60.5	94.3
10	32.3	11.2	34.3	75.2
15	30.0	7.2	13.0	78.0

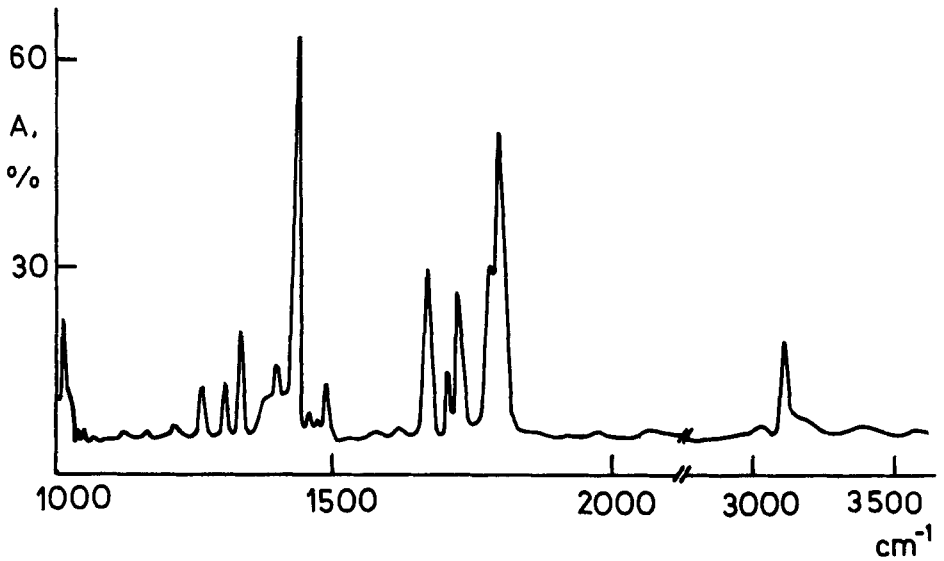


Fig. 1. Infrared spectrum of dithianon in chloroform.

The radiochemical purity of the product is expressed as the percentage of the product activity in the spot on the developed chromatogram with respect to the activity introduced to the start line of the chromatogram. In some determinations of dithianon labelled with a singe radionuclide, the value of the spot activity obtained semiconductographically was verified by liquid scintillation spectrometry of the eluate of the same spot cut out from the chromatogram. The spot activity values determined by the two methods were identical within limits of statistic deviations.

The above exchange procedure was used for the preparation of dithianon ^{14}C, ^{35}S /see Table III/.

As it may be inferred from this Table, the exchange of ^{35}S in dithianon affords higher yields, but the $^{32}S \rightleftharpoons {}^{35}S$ equilibrium is reacked at about 60 % only /i.e..the specific activity of the substance is lower/, on the other hand, the highest possible specific activity is obtained by exchange of ^{35}S in 1,2-dicyanoethenedithiol potassium salt /exchange degree, 99 %/, but the yield of the substance is low beacause of a rapid thermal decomposition of 1,2-dicyanoethenedithiol potassium salt. For this reason, in most cases of a simultaneous labelling with ^{14}C and ^{35}S a direct exchange of ^{35}S in dithianon-^{14}C is recommended since the loss of the ^{14}C radionuclide is lower whereas the disadvantage of the lower specific activity of ^{35}S be decreased by the use of a higher activity of elemental sulphur ^{35}S in the exchange.

TABLE III

Syntheses of Dithianon Labelled with ^{14}C, ^{35}S, and $^{14}C+^{35}S$ by Procedures Stated

Procedure	Labelling	Exchange degree,% $^{32}S \rightleftharpoons {}^{35}S$	Yield,% Radiochem.+	Yield,% Chem.	Radiochem. purity,%
1.	^{14}C	-	-	68.2	99.5
2.	^{35}S /Tab.I/	99.2	17.3	17.4	96.2
3.	^{35}S /Tab.II/	60.0	36.4	60.5	94.3
4.	$^{14}C+^{35}S$ /2above/	98.2	16.3	13.2	97.2
5.	$^{14}C+^{35}S$/3above/	61.2	34.9	39.8	99.2

+ The radiochemical yield was determined in the case of ^{35}S only.

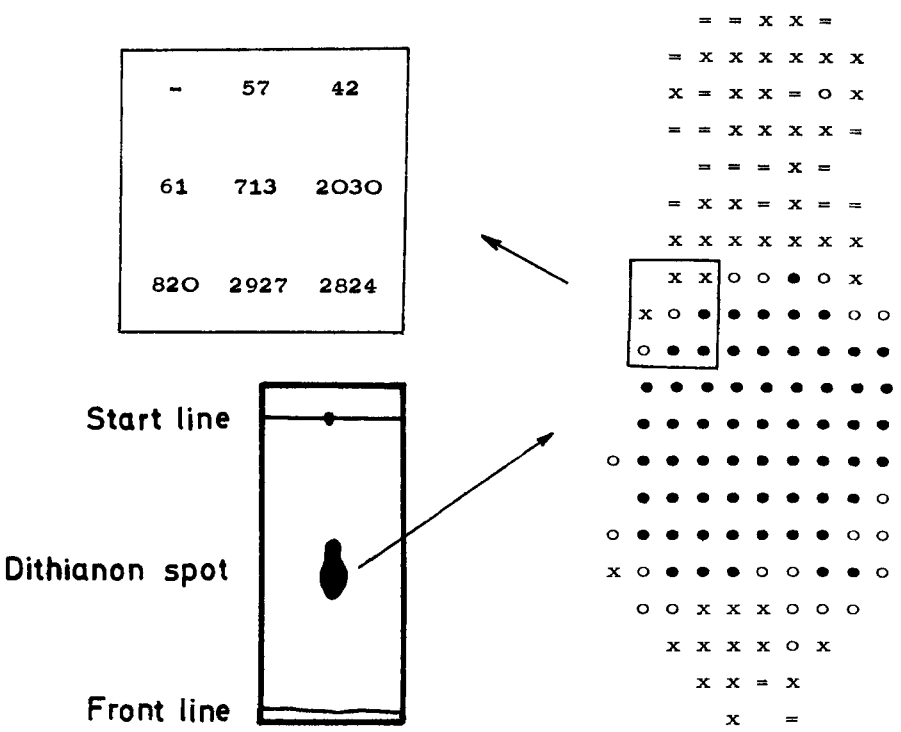


Fig. 2. Semiconductographic analysis in the first counting channel /18-170 keV/ for TLC of dithianon - ^{14}C, ^{35}S: colour record of the active spot with a part of the numerical record. The order of magnitude of the counting rates in the colour record: = /red/ 10^0 , x /green/ 10 , o /black/ 10^2, • /violet/ 10^3.

Fig.2 shows the result of the semiconductographic analysis in the 18-170 keV channel /i.e., for the simultaneous record of $^{14}C+^{35}S$. Dithianon was labelled with both radionuclides and, the next day a sample /1.11 μCi/ of dithianon-^{14}C, ^{35}S was applied to the start line of thin layer of silica gel. The chromatogram was developed in benzene. In addition to a negligible activity on the start line a single active spot was detected /in addition to the colour record of this spot, a portion of the numerical record is also shown on Fig.2/.

The semiconductographic analysis was evaluated from numerical records in both channels in such a manner that the activity of ^{35}S was determined from the observed counting rate in the 156-170 keV channel /see ref.[27]/ while the activity of ^{14}C was calculated[24,28] for each scanned site /i.e., 1 mm^2/ from the known ratio of detection efficiency of ^{35}S in the two channels. Te UV spectrum of the dithianon -^{14}C,^{35}S thus obtained is in accordance with reported data[33] and with the spectrum of the non-active dithianon. The IR spectrum of our product corresponds to that of the non-active counterpart /Fig.1/ but markedly differs from the data of Double[34] that, however, were found to represent an IR spectrum of impure chloroform serving as solvent for the sample of Double.[34]

CONCLUSION

A procedure was developed for the radioisotope labelling of dithianon, a substance of a great applicability in many fields, particularly in protection of agricultural and fruit production.

The labelled substance could be used in investigations on the action mechanism while the doubly-labelled substance could be helpful in metabolisation assays. The radiometrical analysis was effected by semiconductography which makes possible a non-destructive simultaneous determination of ^{14}C and ^{35}S.

The indentity of the product was established by comparison with the non-active standard substance and reported data[34] /m.p., R_F on TLC, UV spectrum and IR spectrum/.

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