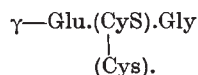


Again three substances which reacted with ninhydrin and which had the R_F values mentioned above were found. Cystine and the intermediate spot were shown by an autoradiograph of the paper chromatogram to contain sulphur-35, while the glutathione disulphide spot did not. By counting the filter paper chromatogram with a thin end-window Geiger-Müller tube, it was found that 30 per cent of the sulphur-35 cysteine was incorporated into the intermediate compound when the initial solution contained equimolar amounts of sulphur-35 cysteine and glutathione.

A similar experiment was carried out with glutathione containing sulphur-35 and non-labelled cysteine. When the sulphur-35 glutathione and cysteine were mixed in solution and the mixture chromatographed, the intermediate disulphide spot again appeared. This substance and glutathione disulphide were shown to be radioactive whereas the cysteine spot was not. It was concluded, therefore, that this material was most probably a mixed disulphide of cysteine and glutathione:



Wikberg² has reported the formation of a mixed disulphide of glutathione and cysteinylglycine when glutathione was partially hydrolysed. His mixed disulphide was separated by paper electrophoresis from cystine and glutathione disulphide. He also reported the preparation of a mixed disulphide of cysteine and glutathione by oxidation with perhydrol at pH 7.5.

The results reported here were discussed before the American Chemical Society Meeting at Los Angeles in March 1953.

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A Method for the Cleavage of 2,4-Dinitrophenylhydrazones and its Application to Steroidal Ketones

2,4-DINITROPHENYLHYDRAZONES have proved very useful derivatives for identification purposes, but have so far found only limited application in preparative work. This is due to the fact that there exists no general method for the regeneration of the corresponding carbonyl compounds. As Djerassi pointed out¹, the failure to cleave 2,4-dinitrophenylhydrazones has imposed considerable limitations on the use of the Mattox-Kendall² dehydrobromination method in the field of sterol chemistry. Thus, in the case of 2,4-dinitrophenylhydrazones of $\Delta^{4,6}$ -dien-3-ketosteroids, only about 5-10 per cent of ketone could be recovered after treatment with pyruvic acid and hydrobromic acid, and derivatives of $\Delta^{1,4}$ -dien-3-ketosteroids could not be split at all¹.

We have now found that the splitting of these 2,4-dinitrophenylhydrazones can be achieved in good yields by the following general procedure, the quantities and times required for saturated-, Δ^1 -, and Δ^4 -3-ketosteroids being denoted by (a), and those for $\Delta^{1,4}$ - and $\Delta^{4,6}$ -3-ketosteroids by (b). The 2,4-dinitrophenylhydrazone (1 gm.) is dissolved in acetone

(a, 200 c.c.; b, 500 c.c.) containing concentrated hydrochloric acid (a, 5 c.c.; b, 10-20 c.c.) and the solution is refluxed (a, 45 min.; b, 7-8 hr.); this displacement can also be brought about at ordinary temperature for 2,4-dinitrophenylhydrazones of class (a) (24 hr.) and for the more soluble derivatives of class (b) (15 days). The yellow to orange-yellow solution is slightly cooled and treated with stannous chloride (5 gm.) dissolved in concentrated hydrochloric acid (20 c.c.). Water (a, 30 c.c.; b, 80 c.c.) is added and the mixture heated to reflux in an atmosphere of nitrogen for 45 min.; further heating decreases the yield and purity of the ketone recovered. With 2,4-dinitrophenylhydrazones of both classes, this reduction can be carried out at ordinary temperature (24 hr.). The acetone is distilled off under reduced pressure at room temperature, the residue extracted with benzene, and the organic layer washed with 1 N hydrochloric acid until no more coloured material is removed. The benzene solution is finally washed with water and evaporated. The recovered ketone is purified by the usual methods.

Using this procedure the following results were obtained with approximately 1 gm. each of 2,4-dinitrophenylhydrazones:

- (1) *Cholestan-3-one*: yield, 98 per cent (m.p., 127-128°; litt., 129-130°).
- (2) Δ^4 -*Cholesten-3-one*: yield, 95 per cent (m.p., 78-79.5°; litt., 80.5°).
- (3) Δ^1 -*Cholesten-3-one*: yield, 84 per cent (m.p., 99-100° (from dil. alcohol); litt., 98-100°).
- (4) *Testosterone*: yield, 98 per cent (m.p., 148-150°; litt., 152°).
- (5) *Progesterone* (via 2,4-dinitrophenylhydrazone): yield, 86 per cent (m.p., 120° (from pet. eth.); litt., 121°).
- (6) Δ^4 -*Cholestadien-3-one*: yield, 83 per cent (m.p., 110-111°; litt., 112°).
- (7) $\Delta^{1,4}$ -*Cholestadien-3-one*: yield, 88 per cent.
- (8) Methyl Δ^1 - Δ^4 -3-ketoetiocoladienate: yield, 67 per cent (m.p., 152-153°; litt., 156°).

As it is somewhat difficult to purify $\Delta^{4,6}$ -cholestadien-3-one on a small scale, the yield was determined by reconvertng it into the corresponding 2,4-dinitrophenylhydrazone (m.p. 234° from acetic acid).

The successful cleavage of the $\Delta^{1,4}$ -dien-3-one derivatives supports Djerassi's assumption that they are true hydrazones and not pyrazoline derivatives (cf. ref. 1, footnote 19, p. 1005).

The final benzene solution of methyl $\Delta^{1,4}$ -3-ketoetiocoladienate was extracted with sodium bicarbonate solution. On acidification this gave 110 mgm. of an acid (m.p. 277-280° with dec.), showing that partial hydrolysis of the ester group had occurred.

Other ketones, such as dibenzyl acetone, have also been recovered successfully by this treatment.

We suggest that the method is of wider application and that it may be useful in other fields of preparative organic chemistry.

We wish to express our thanks to Dr. Carl Djerassi, to the CIBA, S.A. (Basle) and to the Schering A.G. (Berlin) for gifts of specimens which have greatly facilitated this work. Full details of this investigation, including improvements in the preparation and purification of some 2,4-dinitrophenylhydrazones of 3-ketosteroids, will be published in the *Bull. Soc. Chim. Belg.*

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