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REMARKS ON PREPARATION OF INDANDIONE DETECTION REAGENTS

J. Stepan and V. Kral

Translation of: "Poznamky k priprave indandionovych detekcnich cinidel," Casopis Lekaru Ceskych, vol. 92, no. 8, 1953, pp. 208-210.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 AUGUST 1985

STANDARD TITLE PAGE

1. Report No. NASA TM 77905	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle REMARKS ON PREPARATION OF INDANDIONE DETECTION REAGENTS		5. Report Date August 1985
		6. Performing Organization Code
7. Author(s) Jan Stepan and Vaclav Kral		8. Performing Organization Report No.
		10. Work Unit No.
9. Performing Organization Name and Address Leo Kanner Associates Redwood City, CA 94063		11. Contract or Grant No. NASw 4005
		12. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration, Washington, D.C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes Translation of "Poznamky k priprave indandionovych detekcnich cinidel," Casopis Lekaru Ceskych, vol. 92, no. 8, 1953, pp. 208-210.
(UDC 547.665)

16. Abstract A modified Claisen condensation with sliced sodium at a higher temperature is recommended for production of ungranulated charcoal.

A new ninhydrin production method by means of oxidation of benzaldiketohydrinden using available reagents tried by the authors was unsuccessful.

Triketohydrinden was obtained by boiling ninhydrin in acetic acid anhydrides.

17. Key Words (Selected by Author(s))	18. Distribution Statement
	Unclassified-Unlimited

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22.
Unclassified	Unclassified	8	

REMARKS ON PREPARATION OF INDANDIONE DETECTION REAGENTS

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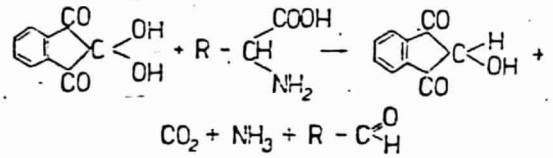
Institute of Biochemistry of Charles University (Plzen Branch)

During a study of vascular system compounds we were confronted with a problem of production of indandione derivatives which are used daily in biochemical laboratories. We wish to present some original data with a brief outline of the problem. /208*

Excess ninhydrin is formed when heating diaminate amino acids according to the reaction scheme:



$$CO_2 + NH_3 + R - C\overset{O}{\underset{H}{<}}$$

Reduced ninhydrin then reacts with ninhydrin and ammonia, forming a blue colored compound, diketohydrinylidendiketohydrindamine, or ammonium salt, according to the reaction scheme [1, 2, 3]: /209

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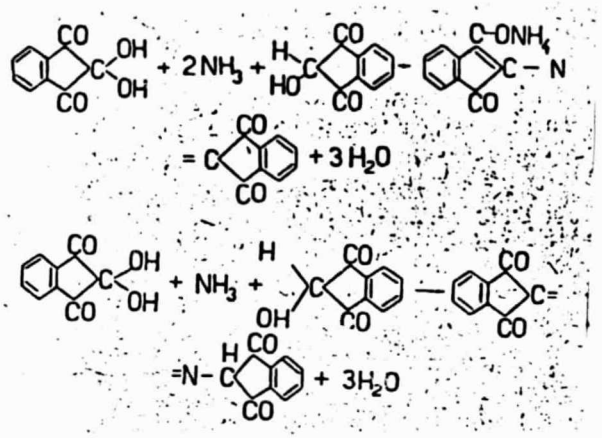


This reaction which is reminiscent of the murexide reaction is used during chromatography detection of amino acids which are important in medicine.

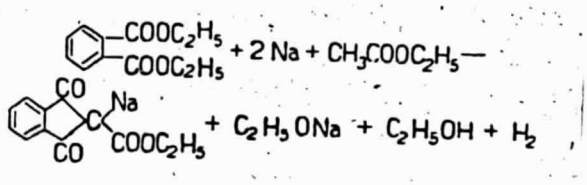
The intermediate reaction product, formed during the production of ninhydrin and diketohydrinden was used by us for qualitative determination of choline in choline salt of nicotinic acid and for chromatography detection [4, 5]. During chromatography a 1% aqueous dioxane solution (1:1) can also be used for choline derivatives providing red to red-violet coloring.

The course of the reaction and the purity of the products were studied during production of choline salt of nicotinic acid from ethylene oxide, trimethylamine and nicotinic acid proper [5]. A reaction resembling amino acids, without adding water, occurred here before the course of the reaction was determined quantitatively.

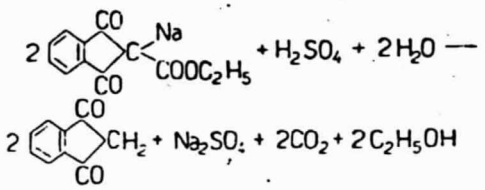
Experimental Part

During production of triketohydrindenhydrates (ninhydrin)

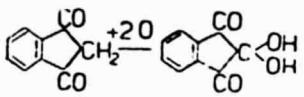
1. By a reaction of diethylphthalate with ethyl acetate and sodium:



sodium salt of ethyl ester-1,3-diketohydrinden-2-carboxylic acid is formed which is then decomposed and undergoes decarboxylation by sulfuric acid:



and undergoes oxidation to ninhydrin by selenium dioxide in an aqueous-dioxane medium:

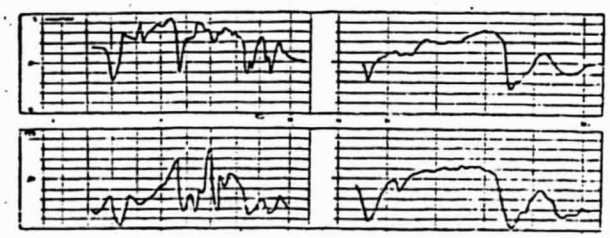


The method of Claisen condensation with powdered sodium, as stated in the literature, entails the disadvantage of a vigorous reaction and an explosion in the laboratory. Using sodium cut into thin slices at a 100-110°C temperature and slow activation of the reaction by adding drops of a mixture of ethyl acetate with anhydrous ethanol for about 90 minutes, we always achieved good results (71% of theoretical yield). During the second reaction the results fluctuated around 99% of the theoretical yield and the end product yield was about 30-33%.

The use of ungranulated charcoal is also important during purification of the end product because colloidal solutions are formed under the effect of even a small amount of granulators, resulting in substantial yield losses. We also recommend addition of selenium dioxide shortly before initiation of the reaction.

Triketohydrinden, a red crystalline compound, was obtained by us by boiling recrystallized ninhydrin with acetic acid anhydrides. We did not find this production method in the literature.

The spectral absorption curves of triketohydrinden and ninhydrides in the infrared region are presented in Figures 1 and 2. A Kipp-Zonen single-beam spectrograph was used for the measurements. The samples were rubbed with vaseline (0.0247 g ninhydrin and 0.1282 g vaseline, 0.0178 g triketohydrinden and 0.1068 vaseline) and measured in a sodium fluoride measuring cell (thickness of vaseline layer 0.025 mm). The size of the inlet and outlet slot was varied from 0.05 mm at λ=2 μ to 0.5 mm at λ=15 μ. The temperature during the measurements was 21°C.



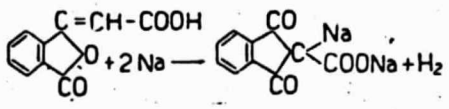
Triketohydrinden (half a band) and ninhydrin absorption curves in infrared region. Wavelengths are plotted on the abscissas and transmittance percentages on the ordinates. A Kipp-Zonen single-beam spectrograph was used.

The elementary analysis was in agreement with the theoretical calculations [1] for both triketohydrin and ninhydrin.

2. The second method is the reaction from phthalic acid anhydride, liquified potassium acetate and acetic anhydride in which phthalyl

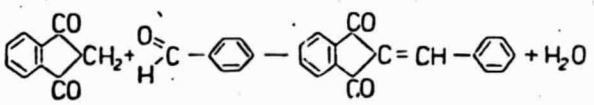
[1]The spectral and elementary analysis were carried out by the Official Authorized Control and Testing Institute in Pardubice-Rybitvi.

acetic acid was obtained with an approximate 47% yield. The latter is converted in a methanol medium to sodium salt of diketohydrinden carboxylic acid



reaction, which is decomposed with hydrochloric acid during boiling with an approximate 48% (theoretical) yield.

3. Benzaldiketohydrin with an approximate 50% pure product yield was obtained by us by condensation of diketohydrin with benzaldehyde:



Benzaldiketohydrinden was purified from chloroform and benzine by means of adsorption, chromatography on an Al_2O_3 layer and recrystallized from methanol. The melting point was 150° (the literature gives 150-151°).

Using a similar method as in a barbiturate ring, we tried oxidation with chromium trioxide. The outcomes of these experiments (also with a Kilian solution, potassium permanganate, red lead, lead peroxide and hydrogen peroxide) were negative.

We explain this by the different structure of the indandione skeleton compared with the barbituric acid skeleton.

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