

Identification of the Main Ethylenediamine Condensate of Noradrenaline with that of Catechol

My attention has been directed to a recent communication¹ by T. Nagatsu and K. Yagi dealing with the identification of the main ethylenediamine condensate of noradrenaline with that of catechol. The authors report some experiments, quote an infra-red spectrum and two microphotographs of crystals. Also, the sentence occurs: "However, the precise identification of these compounds in the pure form has not yet been reported".

In fact, not only "the precise identification of these compounds", but also a rigorous proof of the chemical structure and an independent synthesis of these compounds was reported in a detailed paper by J. Harley-Mason and A. H. Laird more than two years ago², and all the experiments reported by the Japanese authors are contained in this paper.

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¹ *Nature*, 193, 484 (1962).

² *Tetrahedron*, 7, 70 (1959).

THOUGH we missed the report by J. Harley-Mason and A. H. Laird (*Tetrahedron*, 7, 70; 1959), we realize that the results reported in our communication are

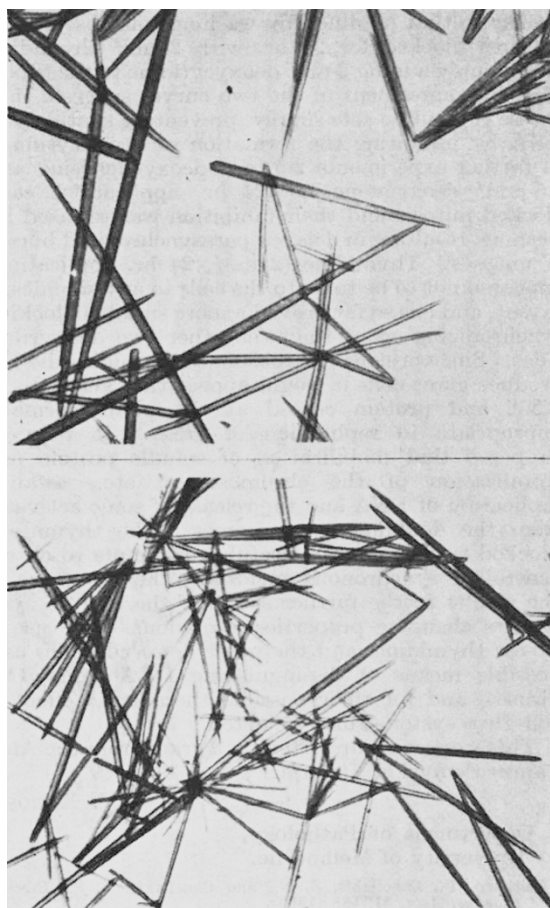


Fig. 1. Picrate crystal of a main ethylenediamine condensate of catechol (above); noradrenaline (below) ($\times 1,000$)

identical with a part of the results reported by them. Therefore, as a result, our communication serves to confirm their results.

We may add another fact confirming the identification of both the main condensates. We isolated both substances as picrates. Both picrates formed similar, needle-shaped, orange red crystals (Fig. 1).

The infra-red spectra of both picrates were identical. The result of elementary analysis of picrate crystals of the main noradrenaline-ethylenediamine condensate was as follows: calc. for $C_{10}H_{16}N_4 \cdot 2C_6H_3N_3O_7$: C: 41.00, H: 2.500, N: 21.74, O: 34.76; found, C: 40.96, H: 2.695, N: 21.67, O: 34.55 per cent.

This result corresponds with the dipicrate of tetrahydro-tetra-aza-anthracene, confirming the result of Harley-Mason and Laird.

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Determination of Free Amino-Acids in Human Ejaculate by Ion Exchange Chromatography

REPORTS on investigations concerning the content of free amino-acids in human ejaculates are rare. Sakar, Luecke and Duncan¹ estimated the amino-acid composition by microbiological methods. Adam and Korting², Jacobsson³ and Lundquist⁴ separated amino-acids from human sperm liquor qualitatively by paper chromatography. Keutel and Gabsch used paper chromatography for qualitative and quantitative determinations of amino-acids from ejaculates⁵.

We analysed quantitatively the content of free amino-acids in sperm liquor by ion exchange chromatography⁶ using an automatic recording equipment⁷. The method is capable of giving reproducible recoveries of better than 100 ± 3 per cent for quantities ranging from 0.25 to 2 μ moles of amino-acid. One complete analysis required 2 days.

Four to six hours after ejaculation, a 10-fold amount of 1 per cent picric acid according to Hamilton's method⁸ was added to the ejaculate. The picric acid precipitate was removed promptly by centrifugation. To remove excess picric acid from the protein-free ejaculate, the solutions were passed through columns of 'Dowex 2' (2 cm. diam.)⁹; a bed 2 cm. in height was used. The colourless effluents were filtered with 'Celite' if cloudy and hydrolysed for 24 hr. with 100 ml. 6 N hydrochloric acid under reflux to split peptides and conjugated amino-acids, if present. The hydrolysate was dehydrated three times in a rotary evaporator, treated with 'Celite', when necessary and made up to a volume of 5-10 ml. with a 0.2 N sodium citrate buffer, pH 2.2¹⁰. All solutions were kept frozen prior to chromatography. An amount of the hydrolysate corresponding to 0.5 ml. of the original ejaculate was chromatographed in most instances. The samples were analysed on columns of 'Amberlite IR-120' using a self-designed amino-acid analyser¹¹.

Table I shows the average values of 10 patients with normospermia, 5 with azoospermia combined with Leydig cell insufficiency and one patient with aspermia due to deformity of accessory sexual glands. Fig. 1 demonstrates a typical effluent curve of free amino-acids from an ejaculate of a case of normospermia.