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REINVESTIGATION OF AUTHENTIC SAMPLES OF AUXINS A AND B, AND RELATED PRODUCTS BY MASS SPECTROMETRY

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Authentic samples of auxin-a, auxin-b and auxin-a lactone — discussed in papers from this laboratory in the period 1931 - 1944 — are found to be identical with cholic acid, thiosemicarbazide and hydroquinone, respectively. Auxin glutaric acid turns out to be phthalic acid, and the 3,5-dinitrobenzoyl ester of auxin-b is ethyl α-cyanophenylpyruvate.

Auxins a and b, as unique individuals, are therefore to be considered as non-existent.

Introduction

The first isolation of a natural plant growth hormone was performed in this laboratory in the years between 1930 and 1935. Human urine was mainly used as a starting material, since plant material contains only very small amounts of the growth stimulating substance.

One of the compounds isolated, chemically characterized as indole-3-acetic acid [1], is now generally recognized as the universal plant growth regulator. However, prior to this isolation other substances called auxin-a and auxin-a lactone were obtained from urine and auxin-b from malt. These compounds, which were stated to lose their biological activity on storage [2], were claimed to be even more active as growth substances than indole-3-acetic acid [3].

Despite very many attempts both in this laboratory and abroad, since 1941 the auxins a and b could never be reisolated. Syntheses of auxin-a and of auxin-b, for which structural formulae had been proposed [4], were never accomplished*.

To get more insight into the nature of these compounds it was decided to reinvestigate the very small samples (1-10 mg) of the authentic products

* *Note added in proof:* A mixture of stereoisomers of auxin-b lactone has recently been synthesized. *M. Matsui* and *Y. S. Hwang*, Proc. Jap. Acad. **42**, 488 (1966).

1 *F. Kögl, A. J. Haagen-Smit* and *H. Erxleben*, Z. Physiol. Chem. **228**, 90 (1934).

2 *F. Kögl, H. Erxleben* and *A. J. Haagen-Smit, ibid.* **216**, 31 (1933).

3 *F. Kögl* and *D. G. F. R. Kostermans, ibid.* **235**, 201 (1935).

4 *F. Kögl* and *H. Erxleben, ibid.* **227**, 51 (1934).

still present in our laboratory, with help of the presently available powerful physical methods. Necessary information about the coded samples was obtained from the original laboratory note books and from published results.

Experimental

Mass spectra were obtained with an A.E.I.-M.S.9 mass spectrometer, using a direct insertion probe. Accurate mass measurements were performed with tris-nonafluorobutylamine as a reference compound. Ultraviolet absorption spectra were measured with a Zeiss PMQ II spectrophotometer. When concentrated sulfuric acid was used as a solvent, the spectra were measured one hour after dissolution. X-ray powder diagrams were recorded with a Debye-Scherrer camera using Cu Kα radiation.

Results

Auxin-a

Auxin-a sample no. 2466 [2] and another, denoted by us as auxin-a L, were examined.

The mass spectra of both preparations are identical and show several peaks of high intensity at masses higher than expected for a substance with the empirical formula $C_{18}H_{32}O_5$. Thus, either the old preparations are heavily contaminated or the structure proposed earlier is not correct. Analysis of the mass spectra (see Fig. 1 and Table I) and accurate mass

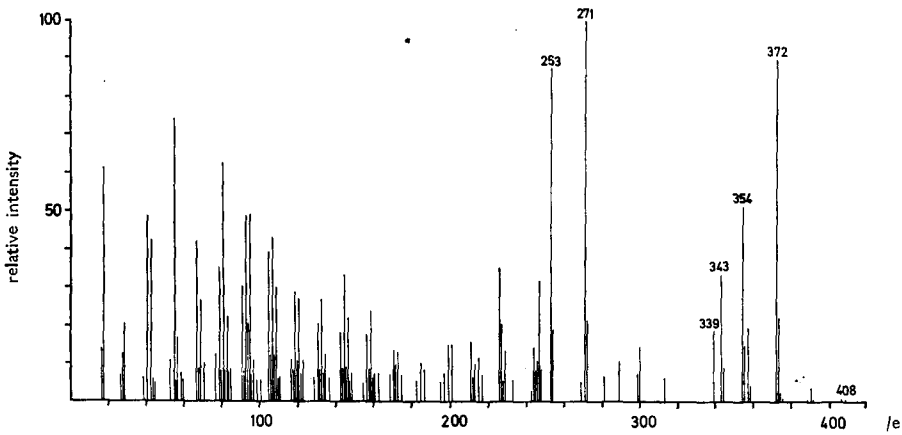


Fig. 1. Mass spectrum of "auxin-a" and of cholic acid. Peaks with a relative intensity lower than 5% have been omitted, except those at m/e > 400.

measurements (Table II) then showed auxin-a to be the C_{24} compound $C_{24}H_{40}O_5$. A comparison with the mass spectrum of cholic acid proved auxin-a to be identical with cholic acid. The identity of the two samples with cholic acid goes even so far, that they contain the same impurity as commercial cholic acid (Hoffmann-La Roche & Co).

Table I

408^+	$\rightarrow$	390^+	+ 18	
390^+	$\rightarrow$	372^+	+ 18	
372^+	$\rightarrow$	357^+	+ 15	
372^+	$\rightarrow$	354^+	+ 18	
372^+	$\rightarrow$	271^+	+ 101	
354^+	$\rightarrow$	300^+	+ 54	and/or $289^+ \rightarrow 271^+ + 18$
354^+	$\rightarrow$	253^+	+ 101	
354^+	$\rightarrow$	339^+	+ 15	
299^+	$\rightarrow$	281^+	+ 18	
271^+	$\rightarrow$	253^+	+ 18	

Interpretation of metastable peaks in the mass spectrum of auxin-a.

Table II

Composition	Calculated mass	m/e Found
$C_{24}H_{40}O_5$ *	408.2876	408.2848
$C_{24}H_{36}O_3$	372.2665	372.2632
$C_{24}H_{34}O_2$	354.2549	354.2556
$C_{23}H_{35}O_2$	343.2637	343.2636
$C_{23}H_{31}O_2$	339.2324	339.2321
$C_{19}H_{27}O$ **	271.2062	271.2053
$C_{19}H_{25}$	253.1956	253.1942

Accurate mass determinations on auxin-a fragments.

* Parent peak.

** Base peak.

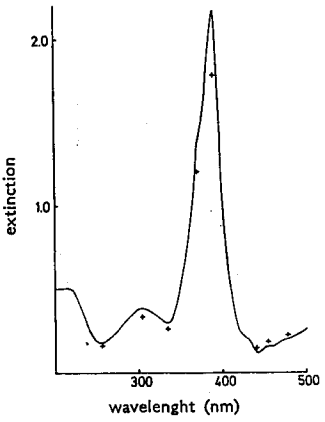


Fig. 2. ———— Ultraviolet absorption spectrum of "auxin-a" in conc. H_2SO_4; ++++++ absorption data [5] of cholic acid in conc. H_2SO_4.

Fig. 2 shows both the absorption spectrum of auxin-a L in concentrated sulfuric acid and the absorption data of cholic acid in the same solvent [5].

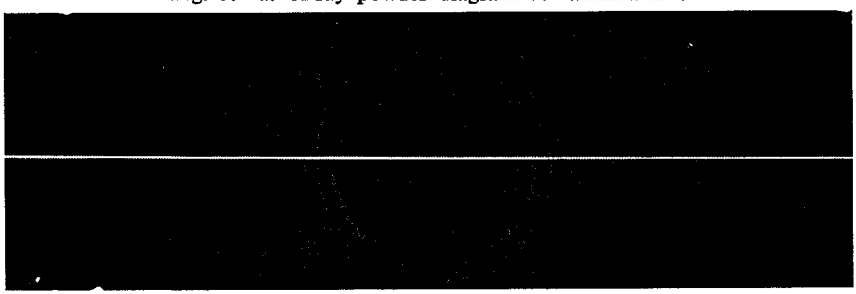
X-ray powder diagrams of auxin-a L and of cholic acid are presented in Fig. 3.

Furthermore, the mass spectra of auxin-a methyl ester (sample no. 1719) and of cholic acid methyl ester were found to be identical.

Auxin-a lactone

Samples no. 1503 [2], no. 8083 [6] and a preparation dated 7-6-'38 were examined. Mass spectral investigation of inactive auxin-a lactone, at the time indicated as lumiauxone [7], proved the molecular weight to be much

Fig. 3. a. X-ray powder diagram of "auxin-a L".

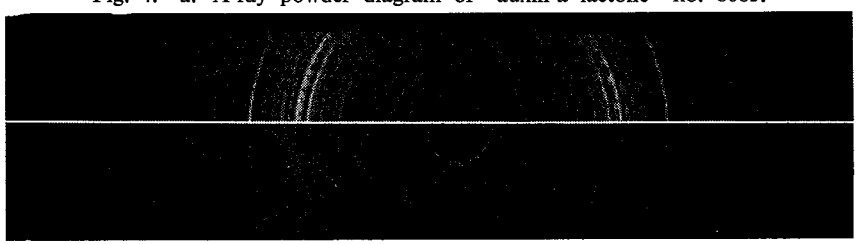


b. *Idem.* of cholic acid.

smaller than originally proposed. The intense peak at m/e 110 was found to be due to $C_6H_6O_2^{\oplus}$. From this empirical formula and the melting-point, 172-173 °C, it could be decided that hydroquinone had been analysed.

Fig. 4 shows the X-ray powder diagrams of sample no. 8083 and of hydroquinone.

Fig. 4. a. X-ray powder diagram of "auxin-a lactone" no. 8083.



b. *Idem.* of hydroquinone.

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[5] *S. Bernstein* and *R. H. Lenhard*, J. Org. Chem. **18,** 1146 (1953).

[6] *F. Kögl* and *G. J. Schuringa*, Z. Physiol. Chem. **280,** 148 (1944); the experiments reported in the Tables V and VI of this paper were performed with sample no. 8083.

[7] *F. Kögl, H. Erxleben* and *C. Koningsberger, ibid.* **280,** 135 (1944).

The ultraviolet absorption spectrum of hydroquinone in ethanol is presented in Fig. 5 together with the spectrum of auxin-a lactone as given in the literature [7].

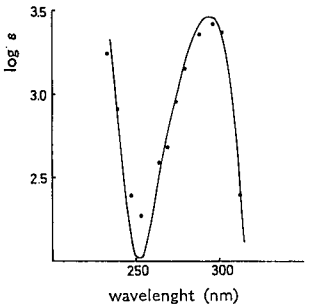


Fig. 5. ———— Ultraviolet absorption spectrum of hydroquinone in ethanol 96%; o o o o o o o absorption data [7] of "auxin-a lactone" in ethanol 96%.

Auxin glutaric acid

Samples nos. 2583, 7246 and 7260 were examined. According to the laboratory note books sample no. 2583 was prepared from auxin-a no. 2466 [2] on 2-12-1932 by oxidation with potassium permanganate. From the same source it was learned that samples nos. 7246 and 7260 showed no melting point depression with synthetic auxin glutaric acid [8, 9].

The mass spectra of the auxin glutaric acid preparations are identical. However they show the molecule to be smaller than $C_{13}H_{24}O_4$, the composition proposed in 1934 [4]. Accurate mass measurements (Table III) together with the actual decomposition point proved the identity of the three samples with phthalic acid. Fig. 6 shows the X-ray powder diagrams of sample no. 2583 and of phthalic acid.

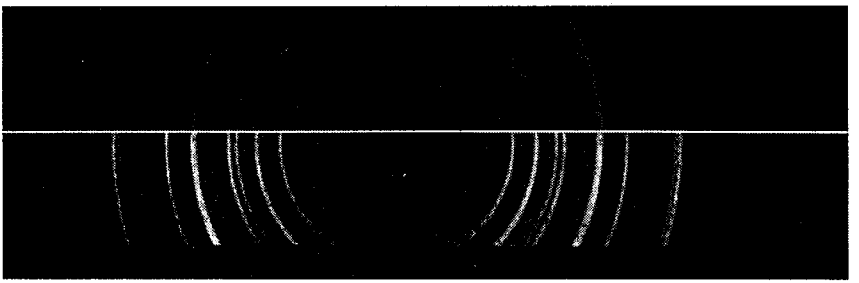
Table III

Composition	Calculated mass	m/e Found
$C_8H_6O_4$ *	166.0266	166.0267
$C_8H_5O_3$	149.0239	149.0231
$C_8H_4O_3$	148.0160	148.0153
$C_7H_6O_2$	122.0368	122.0367
C_7H_4O	104.0262	104.0257

Accurate mass determinations on auxin glutaric acid fragments.
* Parent peak.

[8] *F. Kögl* and *H. Erxleben*, *ibid.* **235**, 13 (1935).
[9] *A. W. Noltes*, Thesis Utrecht 1957.

Fig. 6. a. X-ray powder diagram of "auxin glutaric acid" no. 2583.

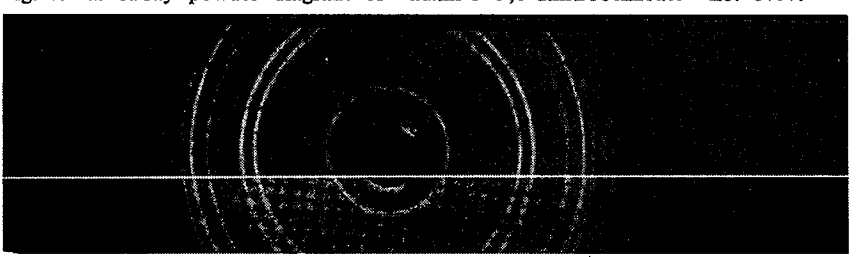


b. *Idem.* of phthalic acid.

3,5-Dinitrobenzoate of auxin-b

Only one sample of this compound, no. 3757, was available. This derivative was studied because it was thought to be of help in disclosing the identity of auxin-b itself. However, the mass spectrum does not show the peaks characteristic for 3,5-dinitrobenzoates [10]. Accurate mass determination provided the empirical formula $C_{12}H_{11}NO_3$ (measured 217.0737, calculated for $C_{12}H_{11}NO_3$ 217.0739). The presumed dinitrobenzoyl ester was actually found to be ethyl α-cyanophenylpyruvate. The X-ray powder diagrams of sample no. 3757 and of synthetic ethyl α-cyanophenylpyruvate [11] are given in Fig. 7.

Fig. 7. a. X-ray powder diagram of "auxin-b 3,5-dinitrobenzoate" no. 3757.



b. *Idem.* of ethyl α-cyanophenylpyruvate.

Auxin-b

Samples nos. 6853 and 7344 were analysed. According to the laboratory note books these samples were isolated from malt. Both samples show the melting point 183 °C (with decomposition with gas evolution) just as reported in the literature [12].

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[10] *K. Biemann*, Mass Spectrometry, McGraw-Hill Book Company Inc., page 190, New York 1962.

[11] Org. Syn. **11**, 40 (1931).

[12] *F. Kögl, H. Erxleben* and *A. J. Haagen-Smit*, Z. Physiol. Chem. **225**, 215 (1934).

The mass spectra indicate that the samples contain sulfur, as the peaks at m/e 32 and 34 are relatively intense. Accurate mass measurements proved this to be true (Table IV). The spectra are temperature dependent; especially the peaks at m/e 131, 116 and 89 tend to be lower at higher temperatures. The peak at m/e 91, which corresponds with $CH_5N_3S^{\oplus}$, is the parent peak of the main component of the two samples, *viz.* thiosemicarbazide. (The behaviour of thiosemicarbazide during melting is

Table IV

Composition	Calculated mass	m/e Found
$C_4H_9N_3S$ *	131.0517	131.0496
CH_5N_3S **	91.0204	91.0197
CS_2	75.9442	75.9439
CH_2NS	59.9908	59.9905
CH_3N_3	57.0327	57.0320

Accurate mass determinations on auxin-b fragments.

* Parent peak acetone-thiosemicarbazone.

** Parent peak thiosemicarbazide.

indeed identical with that mentioned for the two auxin-b samples). The peaks at m/e 131, 116 and 89 belong to acetone thiosemicarbazone $C_4H_9N_3S$, which was found as a more volatile impurity in the two samples. The peak at m/e 76 due to carbon disulfide is a remarkable one. It originates from thiosemicarbazide and it probably results from an ion-molecule reaction.

Discussion

None of the samples investigated has the composition originally proposed. No relation exists between the structures of the auxins, between the structures of the auxins and their derivatives, nor between the structures of the derivatives of the auxins. Thus, auxins a and b are to be regarded, as non-existnt.

The results presented do not offer an explanation for the originally claimed biological activity of auxin-a and auxin-b.

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