REVISTA ESPAÑOLA DE FISIOLOGIA
R. esp. Fisiol., 25, n.º 2, págs. 153-156. 1969

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Sialic acids. XI. On the Thiobarbituric Acid Positive Reaction in Several Materials from the Vegetal Kingdom

The sialic (or acylneuraminic) acids exist only in a few bacteria, but are very widely distributed in the animal kingdom (2, 13, 23). One problem which has called the attention of several authors is the following: Do acylneuraminic acids exist in the vegetal kingdom?

In 1964, MAYER and col. (15) reported the occurrence of a «sialic acid» — not exactly determinated — in soybeans and alfalfa. Also in 1964, CORREL (9) described the existence of a sialic acid containing glycopeptide in the alga *Chlorella*. At the same time we were working on the identification of a product obtained from kidney beans (6) which was positive with the thiobarbituric acid reaction, following the adaptation of the same to the determination of acylneuraminic acids by WARREN (22) and AMINOFF (1). On the other hand, PUSTZAI (20) showed for the first time that hexosamine is found in higher plants as tobacco, kidney beans, etc., as part of glycoproteins; and HUGHES (14) reported the existence of a glucosamine-containing acid substance in plants like mustard and sycamore.

For the hydrolysis, purification, isolation, identification, quantitative determination, etc., we have followed the procedures previously indicated (4, 5, 7, 12), sometimes slightly modified, for example by using «Sephadex» for fractionation, etc.

Our assays with several fractions of kidney beans *(Phaseolus vulgaris)* (6) gave the following results: The purified products isolated from testas and globulins gave a very intense thiobarbituric reaction, the absorption spectra of which was identical to that of the standard of N-acetylneuraminic acid (NANA), as it can be seen in Fig. 1.

The reaction with the embryos was less intense, and with the cotyledons and meal it was negative or gave no typical colors. Other reactions adapted to the acylneuraminic acids, such as the resorcinol-butyl-acetate. Ehrlich (direct), orcinol (Bial) and that of Hestrin for O-acetyl-groups, gave no result. By paper chromatography, one spot was obtained, the Rf of which was near to that of NANA and gave the typical color with the thiobarbituric acid, but not with the other sprays. The reactions for uronic acids, deoxyribose and shikimic acid gave a negative result with the problem.

Also the same happened with the products isolated from lentils *(Lens esculenta)* (6) and sweet almonds *(Amygdalus communis* var. *dulcis)* (6), although the thiobarbituric acid reaction gave less intensity color.

Furthermore, the purified product isolated from the testas of pea seeds *(Pisum sativum)* was also assayed (5). The identity between the chromogen obtained in the thiobarbituric acid reaction and that of

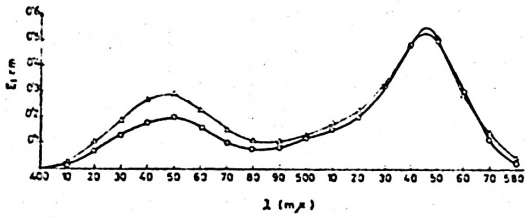

FIG. 1. Absorption spectra of the thiobarbituric acid with NANA, (○); and with the product from kidney beans, (△).

NANA was also confirmed. But, similarly to what happened with the above mentioned materials, the Ehrlich, Bial and resorcinol reactions gave a negative result. Assays (mainly on paper chromatography) for 3-deoxy-L-erythrohexulosonic, muramic, ketobutiric, piruvic and oxalacetic acids, and even with other very different substances as glucosamine, galactosamine, N-acetyl-D-mannosamine, deoxyribose, ribose, adenosine, adenine, histidine, acetaldehide, aspartic and glutamic acids, etc., confirmed that the problem product (which gave one spot with the thiobarbituric acid spray) was not any of those substances; (only the color, but not the Rf, of the deoxy-L-erythrohexulosonic acid was similar; conversely, the Rf was similar to that of the glucosamine).

We have also studied the behaviour of the problem product against neuraminaldolase (8); it seems that it is split by this enzyme, but it does not give N-acetyl-D-mannosamine as it should if it were NANA. Furthermore gas-liquid chromatography was employed, as previously described (4), for the above named product, before and after its attack by neuraminaldolase. It was confirmed that this product, after treatment, was not N-acetyl-D-mannosamine nor N-acetyl-D-galactosamine. Before the enzyme assays the behaviour of the product on gas-liquid chromatography was very different from that of NANA, 3-deoxy-L-erithrohexulosonic and muramic acids; and even more different from osamines and hexoses. The seeds of other vegetals as apricot *(Armeniaca vulgaris)* (5) and maize meal *(Zea mays)* (5) did not give positive results.

The paper of ONODERA et al. (17) reports the occurrence of «sialic acid» —not characterized— in 64 plant materials, amongst which are peels of summer orange and green pepper. We have tried to confirm this result in these two materials *(Citrus aurantium* and *Capsicum annuum)* (5), but unseccessfully; even the thiobarbituric acid reaction did not give, in our hands, the typical color.

Taking into consideration the lack of rigorous specificity of the thiobarbituric reaction —which is the most employed reaction for these assays and which is interfered with several substances as fucose, rhamnose, threonine (24), shikimic acid (16), 3-deoxy-erythrohexulosonic acid, kinic acid, etc. (1, 18, 22), and is produced by 2-keto-3-deoxy-octonic acid (19, 21), etc.— and the fact that its mechanisms with acylneuraminic acids are not well established (3, 10, 18), it seems that it is not correct to assure the existence of acylneuraminic acids in a general manner in the vegetal kingdom. Negative results as those obtained by WARREN (23) corroborate ours. But it is possible that the problem products studied by various authors in the vegetal kingdom could correspond to sialic acids chemically related substances.

Anyway, if sialic acid(s) exist in those plants, it would be in a very small amount. The fact that the product that reacts with

the thiobarbituric acid has been specially detected in some leguminoses could perhaps be explained by the special life of those plants and as a result of the microbiological activity in symbiosis (11), since it is known that some bacteria are able to synthetise a polimer of the N-acetylneuraminic acid, the calominic acid, and a diacetylneuraminic acid (2, 23).

In brief, much attention must be paid before assuring the occurrence of sialic acids in general in higher plants; further works should help to know the nature of the substance(s) which produce, at least in the above materials, a positive reaction with the thiobarbituric acid.

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This work has been made with the help of the Spanish «Ministerio de Educación y Ciencia».

We are grateful to Dr. G. B. PAERELS for his generous gift of 3-deoxy-L-erythrohexulosonic acid.

This paper is part of a lecture given by one of us (J.A.C.) at the Universities of Bochun and Köln (Germany) in October 1967.

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(Received for publication, October 21, 1968)