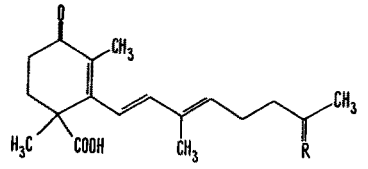
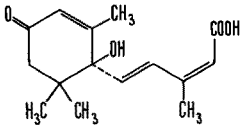
Abscisic Acid-Like Activity of Trisporic Acids

Trisporic acids are sexual hormones of Mucorales[1,2] produced by mixed cultures of plus and minus strains of *Blakeslea trispora*[3,4]. Three different trisporic acids are known, named A, B and C. The structures of trisporic acids B (I) and C (II) have been determined[5-7].



I: R = O; II: R = H, OH

Abscisic acid[8,9] (III) is a plant hormone showing various kinds of activity such as the abscission acceleration of fruits and leaves, the induction of senescence and the prolongation of the dormancy of many seeds.



III

The analogy of structure between abscisic acid and trisporic acids led us to test the latter for their possible activity as plant hormones.

We first tried whether the trisporic acids were able to inhibit the germination of seeds of various species of plants. Because of the low stability of the trisporic acids, we employed the less unstable tetrahydroderivatives[6]. The sample we used was a mixture of Na salts of tetrahydrotrisporic acid A, B and C (about 1–2%, 15% and 80%, respectively).

Seeds of various species were put on a layer of filter paper in Petri dishes, soaked with water solutions of various concentration of the substances and incubated at 24 °C until complete germination was observed with control seeds soaked with plain water. Seeds of the following species were examined: barley (*Ordeum sativum*), bean (*Phaseolus vulgaris*), sesame (*Sesamun indicum*), rape (*Brassica napus oleifera*).

The germination of barley was completely prevented by 1000 µg/ml of tetrahydrotrisporic acids whereas 200 µg/ml caused a decrease of the percentage of germination from 95 to 60 and a delay of about a day. The germination of beans was not entirely prevented at the highest tested dose (2000 µg/ml). There was, however, a delay of 1 or 2 days in germination at concentrations higher than 500 µg/ml. The germination of seeds of sesame was inhibited with 1000 µg/ml, reduced to the 50% with 500 µg/ml and delayed with 100 µg/ml. Seeds of rape, finally, did not germinate at all with 2000 µg/ml and the percentage of germination was 60% with 500 µg/ml.

It is known that the effect of abscisic acid is antagonized by gibberellic acid[10,11]. Therefore a trial was planned in order to put in evidence a possible antagonizing effect of gibberellic acid against tetrahydrotrisporic acids. The results obtained after 5 days' incubation are reported in the Figure.

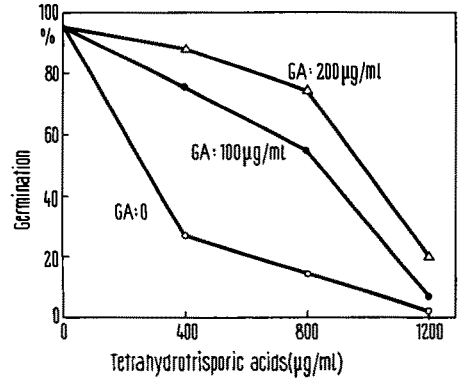
The tetrahydrotrisporic acids determine a strong reduction of germination (with 400 µg/ml it drops to the 27%): they also cause a delay of 1 or 2 days in the germination. Gibberellic acid alone does not determine any increase of the rate of germination but, when administered with the tetrahydrotrisporic acids, it reverses their effect; 200 µg/ml of gibberellic acid practically annul the effect of 400 µg/ml of tetrahydrotrisporic acids. The antagonistic action of gibberellic acid on the tetrahydrotrisporic acids depends on the quantitative ratios between the two substances.

In some experiments on seeds of barley, the Na salts of trisporic acids showed the same inhibiting effect of the tetrahydroderivatives within the first 2 or 3 days of the experiment; later on the effect disappeared. Analyses demonstrated, however, that within 2 or 3 days the trisporic acids, unlike tetrahydrotrisporic acids, disappeared from the solution that soaked the seeds.

It is therefore possible to conclude that both tetrahydrotrisporic acids and trisporic acids exert an action resembling that of abscisic acid: this is more evident with the tetrahydroderivatives because of their higher stability.

As for the doses, these are not very different from those reported for abscisic acid[11]. In this case, in fact, the concentration necessary for inhibiting germination of lettuce seeds was 50 µg/ml.

The results reported strongly suggest that trisporic acids, besides their hormonal activity in the fungi, might exert a regulatory activity in the higher plants[12].



Interaction between tetrahydrotrisporic acids and gibberellic acid (GA) in the germination of barley seeds.

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Riassunto. Gli acidi tetraidrotrisporici e gli acidi trisporici A, B e C inibiscono la germinatione di semi di varie specie di piante. Il loro effetto è antagonizzato da acido gibberellico. Questo fatto e l'analogia strutturale fra gli acidi trisporici e l'acido abscissico suggeriscono che gli acidi trisporici, oltre ad unaattività ormona le nei funghi, possano anche svolgere una attività regolatrice nelle piante superiori analoga a quella esercitata dall'acido abscissico.

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The Identification of εN-(β-Aspartyl)-L-Lysine in Native and Heated Keratin

The presence of an ω-ε isopeptide link between the γ-carboxyl group of a glutamyl residue and the ε-amino group of a lysyl residue has been reported by LORAND et al.[1] and PISANO et al.[2] in polymerized fibrin. Studies on heated keratin indicated that some 'binding' of the ε-amino groups of lysine was occurring[3]. Subsequent work by the present authors, using an enzymic method resulted in the identification and isolation of this moiety in both native[4] and heated keratin[5]. In the case of the heated protein, a greater concentration of γ-glutamyl lysine were found than in the native keratin.

The enzymic method used to isolate this moiety would also leave intact a similar isopeptide link between the β-carboxyl group of any aspartyl residues and the ε-amino groups of lysine. It was observed on amino acid analysis of the keratin digests, that 2 small peaks between leucine and tyrosine did occur (Figure). The slower moving peak was identified as ε(γ-glutamyl) lysine, whereas the more rapid moving peak was suspected to be ε(β-aspartyl) lysine. Subsequent work has now confirmed the identity of this moiety.

Two samples of Merino wool keratin, one of which had been heated for 100 °C for 48 h, were reduced with tributylphosphine, and the thiol groups blocked with acrylonitrile. The resulting proteins were enzymically digested using pepsin, pronase, aminopeptidase M and prolidase as described by COLE et al.[6]. The resulting digests were fractionated by ion-exchange chromatography using the Technicon amino acid analyzer system with stream splitting to moinitor the fractions.

A wide fraction was collected from the column containing leucine, tyrosine, ε(γ-glutamyl) lysine and the unknown. After desalting and concentration this fraction was separated by paper chromatography using butan-1 ol-acetic acid-water (4:1:5 by vol.) as the eluent. By this means the band containing the isopeptide was located and excized. This band was eluted with water and split into 2 fractions, the first portion being hydrolyzed, the second was treated with 1-dimethyl amino-napthalene-5-sulphonyl chloride[7] and then hydrolyzed. The resulting hydrolysates were examined by high voltage electrophoresis and thin layer chromatography. The first hydrolysate showed the presence of aspartic, glutamic acids and lysine thus indicating that as well as the ε(γ-glutamyl) lysine there was also some peptide material containing an aspartyl residue. On analyzing the second hydrolysate the fact that the aspartic acid and lysine are not involved in a normal peptide link was established as DNS-aspartic and DNS-glutamic acids were located along with α DNS lysine thus proving the existance of εN-(β-aspartyl) lysine in wool keratin.

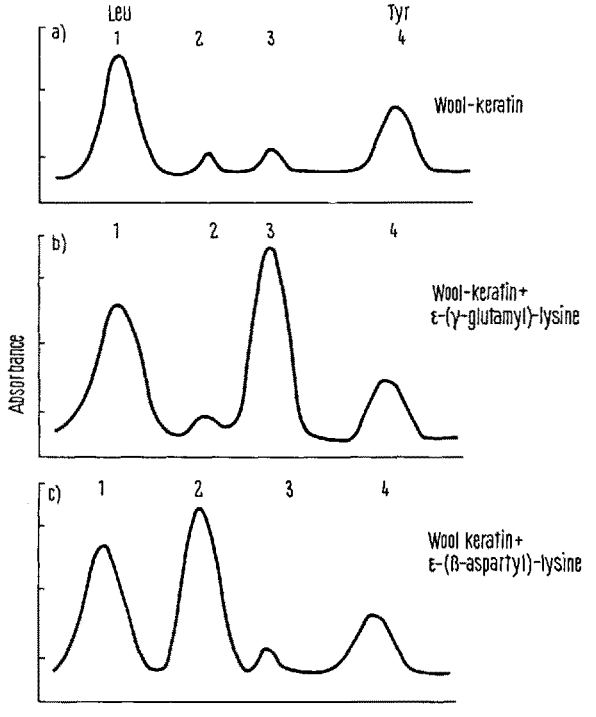
On studying the heated sample of keratin it was found that the amount of εN-(β-aspartyl) lysine had increased (Table).

As a final confirmitory test a sample of synthetic εN (β-aspartyl) lysine was run on the Technicon auto-

Quantities of ε-(β-aspartyl)-lysine in heated keratin

Temperature for 48 h (°C)	ε-(β-aspartyl)-lysine
Control	15
60	17
100	20
120	25

Concentrations in μmoles/g.



Amino acid profiles of wool keratin digests. a) without b) with added ε(γ-glutamyl)-lysine, c) with added ε(β-aspartyl)-lysine. 1. leucine. 2. ε(β-aspartyl)-lysine. 3. ε(γ-glutamyl)-lysine. 4. Tyrosine.

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