

Squaric-acid-diethylester - a strong sensitizer

U. NOSTER, B. M. HAUSEN, B. KRISCHE AND K. H. SCHULZ

Department of Dermatology, University Hospital, Hamburg and
Institute of Organic Chemistry, University of Hamburg, Hamburg, Germany

A patient developed a circumscribed contact dermatitis after a single contact with squaric-acid-diethylester (SADE). Patch tests with low concentrations of SADE were positive. Based on these observations, experimental studies on the sensitizing capacity of SADE were performed on guinea-pigs. The results disclosed that this compound is a strong sensitizer. Derivatives of SADE, such as the nearly insoluble squaric acid, squaric-acid-1,2-diamide and dithio-N,N'-dicyclohexyle-1,2-diamide were not able to produce allergic skin reactions in the sensitized patient nor in guinea-pigs. The chemistry and the range of uses of squaric acid and its derivatives are briefly mentioned. A hypothesis on the molecular mechanism of conjugation of SADE to human proteins and thereby on sensitization is discussed. A warning is given for taking precautionary measures before handling this substance.

Key words: allergic contact dermatitis - guinea-pig sensitization - skin tests - squaric-acid-diethylester.

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Contact allergy to chemicals which are to be used industrially is seldom recognized before the chemicals enter the market.

We would like to report a case caused by a substance which was first discovered in 1959.

Case Report

A chemistry student who had been in contact for one day with squaric acid and its derivatives, especially with squaric-acid-diethylester (SADE) (Fig. 1) developed an area of sharply defined redness on his right cheek. The patient had never been in contact with this compound before, so that this reaction had to be regarded as a toxic one. With a topical corticosteroid the symptoms healed rapidly. About one week later a relapse developed though no fresh contact with the material had occurred. Erythema,

slight swelling and vesiculation could be seen in the previously affected area which disappeared within ten days (Fig. 2).

After complete recovery, epicutaneous tests were carried out with the compounds with which he had been in contact. The results are summarized in Table 1.

No reaction could be obtained to the insoluble compounds such as squaric acid, squaric-acid-1,2-diamide and dithio-N,N'-dicyclohexyle-1,2-diamide. However, application of various concentrations of squaric-acid-diethylester (SADE) gave strong skin reactions.

Control tests performed on ten volunteers under the same conditions proved to be negative. However, one of these volunteers returned 14 days later with evidence of an inflammatory reaction in the region of the test area. He reported having noticed redness

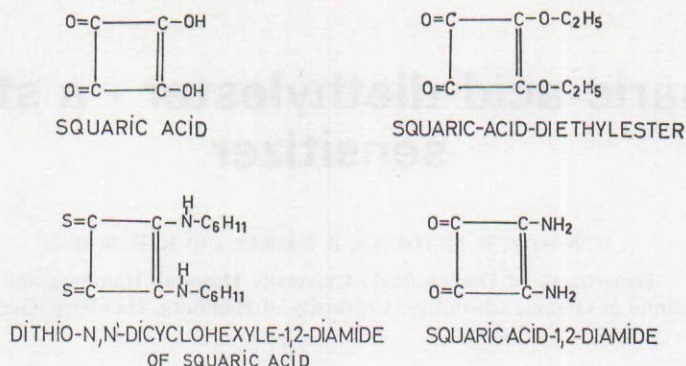


Fig. 1. Squaric acid and its derivatives.

and itching 6–8 days after the compound (SADE) had been applied.

The course of the patient's contact reactions as well as the observations made on this control person indicated that the second reaction which developed without any further contact of the suspected compound had to be regarded as a flare-up phenomenon.

In order to obtain further details of the sensitizing potency of SADE, experiments were performed on guinea-pigs.

Material, Animal Experiments and Results

Determination of the irritancy threshold

In order to determine the toxic threshold

of SADE, 0.05 ml of different concentrations in ethanol (96 %) were applied once to areas of 2–3 cm² of the clipped and shaved flanks of normal guinea-pigs. Twenty-four and forty-eight hour readings revealed that the irritancy threshold lay between 1 and 3 %: a 3 % solution produced positive skin reactions (++) whereas the 1 % concentration was without effect.

Sensitization by multiple applications of non-toxic concentrations

The experiments were carried out on 15 Albino guinea-pigs of the Pirlbright white strain. The animals weighed between 500 and 700 grams. They were fed with Altramin (mixed feed) and water in unlimited amounts.

0.05 ml of a 1 % SADE solution in ethanol was applied daily (except at weekends) to the clipped and shaved flanks (2 cm²). This treatment was continued until strong reactions (+++) had developed (2–3 weeks). Three weeks later, decreasing concentrations of SADE (1 %, 0.3 % and 0.1 %) were applied once to the opposite flanks in order to elicit the allergic reactions. The results are summarized in Table 2.

When challenged with a 1 % solution, all animals showed distinct or strong reactions at



Fig. 2. Circumscribed dermatitis of the right cheek after contact with squaric-acid-diethylester.

Table 1. Results of epicutaneous tests on the patient.

Compound	Conc.	24 h	48 h	72 h
squaric-acid-diethylester	0.1 % A	+++	+++	++
squaric-acid-diethylester	0.03 % A	+++	++	++
squaric-acid-diethylester	0.01 % A	+++	++	++
squaric acid	0.1 % W	Ø	Ø	Ø
squaric acid	0.03 % W	Ø	Ø	Ø
squaric acid	0.01 % W	Ø	Ø	Ø
dithio-N,N'-dicyclohexyle-1,2-diamide of squaric acid	0.1 % W*	Ø	Ø	Ø
dithio-N,N'-dicyclohexyle-1,2-diamide of squaric acid	0.03 % W*	Ø	Ø	Ø
dithio-N,N'-dicyclohexyle-1,2-diamide of squaric acid	0.01 % W*	Ø	Ø	Ø
squaric-acid-1,2-diamide	0.1 % W*	Ø	Ø	Ø
squaric-acid-1,2-diamide	0.03 % W*	Ø	Ø	Ø
squaric-acid-1,2-diamide	0.01 % W*	Ø	Ø	Ø

(A = ethanol; W = water)

* Suspension

a 24 hour reading. The 0.03 % concentration produced definite reactions in 33 % of the animals, weak reactions in a further 33 % and no responses in the remaining 33 % (24 hour reading). The 0.1 % concentration gave negative results with only a few exceptions (3 out of 12 animals).

Sensitization by a single application of toxic concentrations

0.05 ml of a 3 % SADE solution in ethanol was applied to the skin of five guinea-pigs (open epicutaneous method) and the same amount of a 10 % SADE solution to a further five animals.

Table 2. Results of challenge with different concentrations in guinea-pigs sensitized to squaric-acid-diethylester (SADE) in ethanol.

Guinea-pig No.	SADE 1 %		SADE 0.3 %		SADE 0.1 %	
	24 h	48 h	24 h	48 h	24 h	48 h
1	++	++	++	(+)	+	(+)
2	+	++	(+)	Ø	(+)	Ø
3	++	++	(+)	(+)	(+)	Ø
4	+	(+)	Ø	Ø	Ø	Ø
5	++	+	(+)	(+)	Ø	Ø
6	++	+	++	+	Ø	(+)
7	+	(+)	+	(+)	(+)	Ø
8	++	+	Ø	Ø	Ø	Ø
9	+	+	Ø	Ø	Ø	Ø
10	++	++	Ø	Ø	Ø	Ø
11	++	++	(+)	(+)	Ø	Ø
12	++	++	+	(+)	+	(+)
13	+	+	+	(+)	Ø	Ø
14	++	++	+	(+)	(+)	Ø
15	+	+	(+)	Ø	Ø	Ø

Table 3. Test reactions to different concentrations of SADE in guinea-pigs sensitized by one single application of 0.05 ml SADE 3 % and 0.05 ml SADE 10 % in ethanol

Sensitized with	Guinea-pig No.	24 hours			48 hours		
		1 %	0.3 %	0.1 %	1 %	0.3 %	0.1 %
3 %	1	+	Ø	Ø	+	(+)	Ø
	2	+	Ø	Ø	+	(+)	(+)
	3	++	+	(+)	++	+	(+)
	4	+	(+)	Ø	++	(+)	Ø
	5	++	(+)	Ø	++	(+)	Ø
10 %	I	++	+	Ø	++	(+)	Ø
	II	+	(+)	Ø	+	(+)	Ø
	III	(+)	Ø	Ø	(+)	Ø	Ø
	IV	+	(+)	Ø	+	(+)	Ø
	V	+	Ø	Ø	+	Ø	Ø

Challenge after ten days with a 1 % concentration showed at the 24 and 48 hour readings that all five animals sensitized with 3 % SADE gave positive reactions from + to +++, whereas the 0.3 and 0.1 % concentrations produced appreciably weaker reactions. The skin responses with the 10 % solution were of the same order (Table 3).

Since no reactions had been observed in the patient after epicutaneous testing with pure squaric acid or its above-mentioned derivatives (amides), these compounds were tested in concentrations decreasing from 2.0 to 0.3 % on the guinea-pigs sensitized with SADE. For this purpose, the substances were sealed to the flank by an elastic bandage for 24 hours (occlusive technique – closed epicutaneous method of Magnusson and Kligman (1970). As the compounds were difficult to dissolve, therefore water, ethanol and petrolatum were tried as ve-

hicles by making suspensions. No reactions could be obtained with any of the three preparations.

Squaric Acid and its Derivatives

Squaric acid (1,2-dihydroxycyclobutenedione) is a colourless, odourless crystalline substance (Fig. 3). It is insoluble in the usual organic solvents. It can be dissolved up to 2 % in water at 20°C and 11 % at 100°C.

Squaric acid can be described as a strong dibasic proton acid. It was first synthesized in 1959 by Cohen, Lacher and Park. Today its synthesis starts from hexachloro-butadiene, which is obtained as an unwanted by-product during the technical production of perchlorinated hydrocarbons (Maahs & Hegenberg 1966).

The interest aroused by squaric acid is accounted for by the fact that the dianion of this compound can be regarded as a member of a series of new kinds of aromatic compounds (Sprenger & Ziegenbein 1968).

With alcohols, squaric acid forms the corresponding squaric-acid-esters, which can easily be transformed into the corresponding amino compounds (1,2-diamides) (Maahs & Hegenberg 1966).

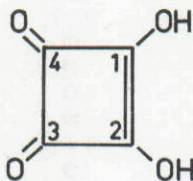


Fig. 3. Squaric acid (1,2-dihydroxy-cyclobutenedione).

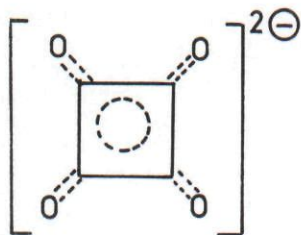


Fig. 3a. Dianion of squaric acid.

Squaric-acid-diethylester (SADE) is a light brown-yellow liquid which is readily soluble in ethanol and other organic solvents. When exposed to atmospheric moisture it undergoes hydrolysis within a few hours producing squaric acid.

A considerable number of new types of coloured compounds can be obtained by means of condensation reactions of squaric acid or its esters with nucleophile pyrrols, phenols, derivatives of malonic acid or similar compounds (Gauger & Manecke 1969). The reaction of squaric acid or its esters with bifunctional primary or secondary amines results in polyamides possessing useful mechanical and thermal properties (Treibs & Jacob 1965).

Two U.S. patents exist which deal with the use of squaric acid and SADE as stabilizers and antifog agents in photographic emulsions (Beavers 1969, 1971).

Though these promising results of basic research are obviously valuable, squaric acid and its derivatives are to our knowledge not yet utilized industrially.

From the literature we found only one paper on the biological properties of squaric acid. Squaric acid was tested for carcinogenic activity: it caused a low incidence of tumors at the injection site in mice (van Duuren et al. 1971).

However, the producer (Hüls AG in the Federal Republic of Germany) indicates in its leaflets as well as on the labels the possibility of an irritating effect on the skin.

Discussion

Clinical observations on humans as well as sensitizing experiments on guinea-pigs have shown squaric-acid-diethylester to be a strong sensitizer comparable to DNCB (1,3-dinitro-4-chlorobenzene) or chloromethylimidazoline (Schulz & Schöpf 1967). Guinea-pigs can be rapidly and easily sensitized by repeated epicutaneous application of 1 % SADE solution which is not primarily irritant to the skin.

However, a single application of a 3 % or 10 % solution (in ethanol) will be enough to sensitize guinea-pigs.

In contrast, squaric acid itself and its two amino-derivatives (diamide and dithioamide) are, if at all, considerably less effective.

SADE can be regarded as a hapten which conjugates with a protein to form an antigen. Hypothetically, the mechanism of this reaction can be approximately demonstrated as follows: having penetrated into the skin, conjugation with free amino groups of proteins takes place leaving the complete antigen and ethanol (Fig. 4).

This hypothesis becomes plausible from the results of Whitfield and Friedman (1972) who tried to modify wool by means of SADE in a mixture of dimethyl sulfoxide

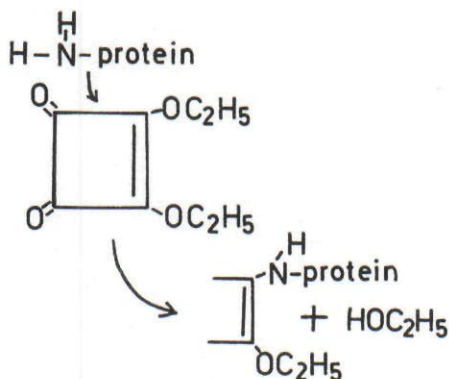


Fig. 4. Hypothetic conjugation of SADE to human protein, forming a complete antigen.

and triethylamine. They found no noticeable change in the physical properties of the wool fabrics, but the aminoacids arginine and lysine appeared to be attacked and modified by SADE.

Industrial use of squaric acid and its derivatives is evidently not yet common. Experiments with these compounds are at present still limited to laboratories. Nevertheless it seems reasonable, in view of the results described here, to issue a warning against repeated contact with SADE, since a single application is sufficient to induce sensitization.

This warning must be emphasized all the more, since SADE is an irreplaceable intermediate product during the synthesis of most 1,2-derivatives of squaric acid.

If our hypothesis is correct there will be no escape from the hazard by taking other esters instead of the diethylester, since they will always react in the same way.

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Address:

Udo Noster, M. D.
Department of Dermatology
Division of Allergology
University Hospital
Martinistr. 52
2 Hamburg 20
Germany

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