

Letter to the Editor

Contact allergy to capryloyl salicylic acid: a mechanistic chemistry and structure–activity perspective

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Dear Sir,

de Groot et al. have reported contact allergy, in 2 patients, to capryloyl salicylic acid (5-CSA, **1**, CAS no. 78418-01-6), also known as 2-hydroxy-5-octanoylbenzoic acid, which is used as a skin conditioner in various cosmetic products (1). The structure is shown in Fig. 1.

Here, we consider whether 5-CSA is likely to be a contact allergen in its own right, or whether the reported allergy is more likely to be to a by-product of its manufacture.

The chemical principles of skin sensitization have been, and continue to be, quite extensively investigated. The fundamental mechanistic basis of structure–activity relationships for sensitization is that, for a chemical to sensitize it must be reactive, i.e. able, either as such or after *in cutaneo* activation, to covalently modify the structure of cutaneous proteins or peptides (2–5).

5-CSA has a very simple structure, and the only plausible way in which it could react covalently with a protein is by the Schiff base mechanism, which involves a protein nucleophile attacking the carbonyl group in the 5-position. Is this carbonyl group reactive enough? The Schiff base mechanism for skin sensitization has been investigated with quantitative mechanistic modelling (QMM) (3) and experimental chemistry (6) approaches. The overall position can be summarized as follows.

Simple aldehydes are reactive enough to sensitize. Their potency, at least in the murine local lymph node assay (LLNA), depends on the combined electronegativity of the groups bonded to the carbonyl group, which can be modelled by the sum of their Taft substituent constants $\Sigma\sigma^*$, together with their hydrophobicity, which can be modeled by $\log P$, where P is the octanol/water partition coefficient (3). Taft σ^* values have been determined experimentally and compiled (7) for a large number of

chemical groups. Where experimental σ^* values are not available, σ^* can often be estimated with the methods described by Perrin et al. (7).

Simple ketones are not reactive enough to sensitize. This is reflected in the lower values of $\Sigma\sigma^*$ for the ketones RCOR' than for the aldehydes RCHO – a difference of 0.49 when R' is methyl, and a larger difference when R' is a higher alkyl (7). However, ketones activated by another group (e.g. a second carbonyl group in the α position or β position) can be reactive enough to sensitize.

Many aromatic aldehydes are non-sensitizers and, in particular, a hydroxyl group in the *para* position has been found to be deactivating. Thus, Natsch et al. (6) found negligible reactivity towards model peptides for the non-sensitizer *p*-hydroxybenzaldehyde.

Bearing in mind the deactivating effect of the *para* hydroxyl group, and the generally lower potency of ketones than of aldehydes, it seems unlikely that the contact allergy observed for 5-CSA is the result of 5-CSA sensitizing by the Schiff base mechanism. It is relevant at this point to note that the European Chemical Agency database (8) contains details of two guinea-pig maximization tests with substantially different results (Table 1). This is consistent with the interpretation that 5-CSA is not itself a sensitizer, but can contain an allergenic impurity, at levels that can vary from sample to sample, that is responsible for the sensitization observed.

It is often the case that the level of a sensitizer required to elicit a response in an already sensitized individual is substantially lower than the level required to sensitize (9), and bearing this in mind we can envisage a scenario whereby an individual becomes sensitized by exposure to a product containing a relatively high level of a allergenic impurity, and can subsequently react to the same product even with much lower levels of the sensitizing impurity.

We now consider how a sensitizing impurity might be present in 5-CSA as a by-product of the manufacturing process.

The synthesis of 5-CSA is described in a patent published in 1989 (10). We assume that the manufacturing process is similar to the synthetic method (Fig. 1), which is based on Friedel–Crafts acylation of methyl salicylate by capryloyl chloride and aluminium trichloride to

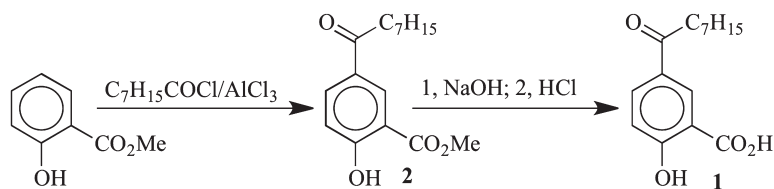


Fig. 1. Synthesis of 5-CSA (1).

Table 1. Guinea-pig maximization test data for 5-Capryloyl salicylic acid (5-CSA)

	Test 1	Test 2
Date of test	1989	1993
Purity data	Not provided	Not provided
Injection induction (%)	1	0.5
Topical induction (%)	0.5	10
Challenge (%)	2	2
Vehicle	Arachis oil	0.5% aq. carboxymethylcellulose for injection induction; ethanol for topical induction and for challenge
Results, 24 hr	14/20	5/20
Results, 48 hr	4/20 + desquamation in 8 others	5/20

give **2**, the methyl ester of 5-CSA. This ester is hydrolysed with sodium hydroxide, after which acidification gives 5-CSA (**1**).

The two most obvious potential side reactions are as follows:

O-acylation, in which the phenolic OH group is esterified, the $-\text{COC}_7\text{H}_{15}$ group becoming bonded to the phenolic oxygen atom. This would lead to the by-product **3** shown in Fig. 2, the methyl ester of *o*-octanoyloxybenzoic acid (**4**). Both **3** and **4** are phenolic esters, and as such are likely to be reactive enough to sensitize by the acyl transfer mechanism (3, 4). However, we consider it unlikely that either **3** or **4** is responsible for the contact allergy observed by de Groot et al. (1), for two reasons. First, the aluminium trichloride-catalysed *O*-acylation reaction is reversible, so although **3** may be formed early in the process, and indeed is probably the initial reaction product, under the Friedel–Crafts reaction conditions it will be converted to the more thermodynamically stable C-acylation products **2** (Fig. 1) and **5** (Fig. 3). Second, as phenolic esters are more easily hydrolysed than alkyl esters, the $-\text{OCOC}_7\text{H}_{15}$ group is unlikely to survive the second stage of the process, in which the $-\text{CO}_2\text{Me}$ group is hydrolysed. Instead, any **3** remaining in the reaction mixture after the first step would be hydrolysed to sodium caprylate and methyl salicylate, the latter being in turn hydrolysed to methanol and sodium salicylate.

C-acylation in the 3-position. In electrophilic aromatic substitution reactions, such as the Friedel–Crafts reaction, aromatic $-\text{OH}$ groups are *ortho/para*

directing and activating, whereas aromatic $-\text{CO}_2\text{Me}$ groups are *meta* directing and deactivating. Consequently, in methyl salicylate, the 3-position (*ortho* to $-\text{OH}$ and *meta* to $-\text{CO}_2\text{Me}$) and the 5-position (*para* to $-\text{OH}$ and *meta* to $-\text{CO}_2\text{Me}$) are the most reactive. The *ortho/para* ratio in electrophilic aromatic substitution reactions depends on several factors (11, 12) – steric considerations favour higher reactivity at the *para* position, complexation or coordination between the electrophile and the activating group can favour higher reactivity at the *ortho* position, and, because of the reversible nature of the reaction, the *ortho/para* ratio can vary according to whether the reaction is carried out under kinetic control or thermodynamic control conditions. In the present case, because of the possibility of intramolecular hydrogen bonding, the *ortho*-substitution product may be thermodynamically favoured.

Particularly relevant is a paper by Ralston et al. (13) on the acylation of phenol (related to methyl salicylate by the absence of the $-\text{CO}_2\text{Me}$ group) with capryloyl chloride and aluminium trichloride. In a series of 18 experiments in which the proportions of reagents, the order of addition, the reaction time and the temperature were all varied, the yields of *ortho* and *para* reaction products were determined. From these data, the apparent reactivity at a single *ortho* position can be compared with that at the *para* position (ratio of *ortho* product to *para* product, divided by 2 to allow for the fact that, in phenol, there are two available *ortho* positions and one *para* position). These values range from 0.12 to 1.39. If these values were applied to the reaction of methyl salicylate with capryloyl chloride,

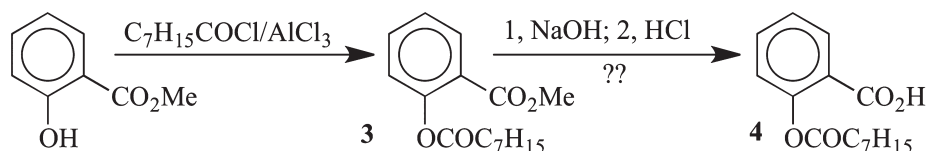


Fig. 2. O-acylation as a possible side reaction in 5-CSA synthesis.

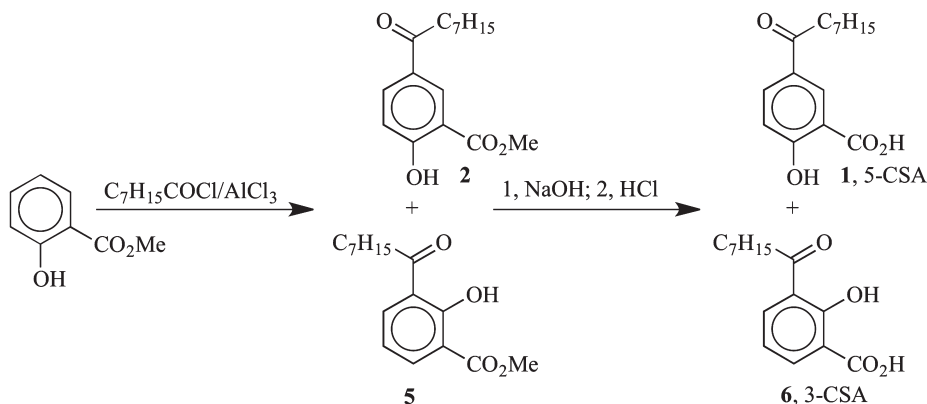


Fig. 3. Synthesis of 5-CSA showing formation of 3-CSA.

Table 2. Predicted aromatic H chemical shifts (ppm) in 3-Capryloyl salicylic acid (3-CSA) and 5-Capryloyl salicylic acid

3-CSA	5-CSA	Easily detectable difference?
7.19 (position 5)	7.24 (position 3)	No
7.68 (position 4)	7.93 (position 6)	Yes
8.22 (position 6)	8.14 (position 4)	No

the amount of 3-Capryloyl salicylic acid (3-CSA) formed would range from 11% to 58% of the total 3-CSA plus 5-CSA.

Overall, therefore, from consideration of the literature on electrophilic aromatic substitution (11–13), we expect significant quantities of 3-CSA (**6**) (> 10% of the total of 3-CSA plus 5-CSA) to be formed, via its methyl ester **5**, in the synthetic route, as shown in Fig. 3.

Some of the 3-CSA may possibly be lost in the post-reaction work-up stages of the synthesis, but we consider it unlikely to be completely absent unless it has been specifically targeted to be removed. We note that, because 3-CSA and 5-CSA are closely related isomers, they are not distinguishable from each other by elemental analysis or mass spectrometry. We have used the nuclear magnetic resonance (NMR) simulation facility in CHEMDRAW[®] (<http://www.cambridgesoft.com/>) to compare the theoretical proton NMR spectra of 5-CSA and 3-CSA. The two are very similar, with slight differences in the shapes and positions of the signals corresponding to the three aromatic protons (Table 2).

The presence of 3-CSA in a sample of 5-CSA would, according to the CHEMDRAW[®] prediction, result in an apparent broadening of the peaks at 7.24 and 8.14 and the appearance of a new peak at 7.68. We consider that, unless the presence of 3-CSA is specifically looked for, at up to ca. 10% it could easily go undetected.

Our conclusion so far is that 3-CSA is a highly plausible impurity in 5-CSA. We now need to consider whether 3-CSA is likely to be allergenic.

As already stated, a hydroxyl group in the *para* position deactivates aromatic aldehydes towards Schiff base formation and sensitization by the Schiff base mechanism, and the same is likely to apply to aromatic ketones. However, the opposite is the case for a hydroxyl group in the *ortho* position. Thus the strong sensitizers atranol (**7**) and chloroatranol (**8**) have hydroxyl groups in both *ortho* positions relative to the aldehyde group, and salicylaldehyde (**9**), with one hydroxyl group *ortho* to the aldehyde group, is a human sensitizer (no potency data). Unlike aromatic aldehydes lacking an *ortho*-hydroxyl group, these three aldehydes show high reactivity in an aqueous lysine-peptide assay (6). The *ortho*-hydroxyl group enables the initially formed Schiff base derivative (**10**) to rearrange to a water-stable enaminone tautomer (Fig. 4), and this has been argued to be the basis of the high potency of these *ortho*-hydroxy aldehydes. We note that the enamine tautomer (**11**, **12**), although not obviously so from the structure **11** shown in (6), is aromatic (**12**; Fig. 4).

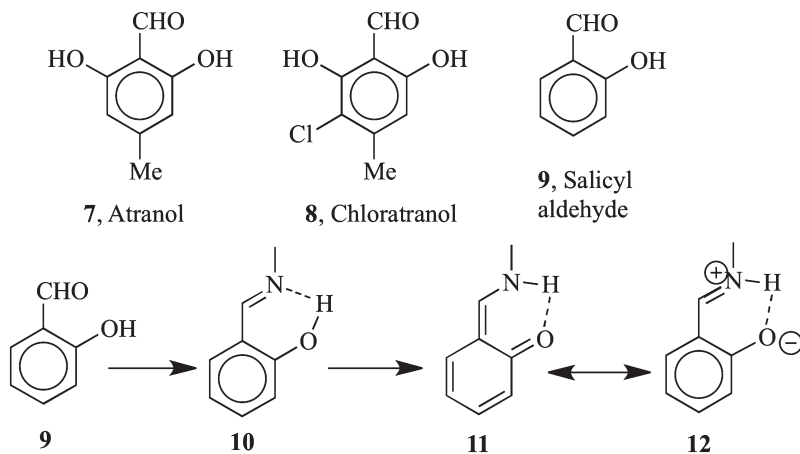


Fig. 4. *Ortho*-hydroxy aromatic aldehydes and their reaction (illustrated with salicyl aldehyde) with the amino group.

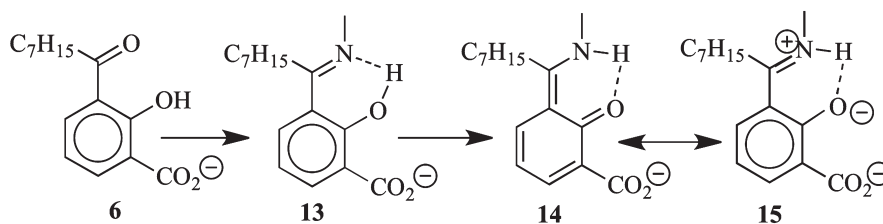


Fig. 5. Proposed chemical mechanism for sensitization by 3-CSA (6).

Note that **11** and **12** are different representations of the same chemical. The double-headed arrow between **11** and **12** signifies that the bonding is intermediate between that represented by **11** and that represented by **12**.

In the same way that an *ortho*-hydroxyl group enhances Schiff base reactivity and sensitization potency for aromatic aldehydes, we would expect that it would enhance reactivity and sensitization potency for aromatic ketones. Thus, although 5-CSA is unlikely to be reactive enough to sensitize, it is quite plausible that 3-CSA could be a sensitizer, reacting as shown in Fig. 5.

The carboxyl group is shown ionized, as the carboxylate anion, as it would predominantly be at physiological pH.

Illustrating how physical organic chemistry provides chemists with useful tools for evaluating hypotheses of the type presented here, we can make an assessment of the plausibility of 3-CSA being the sensitizer responsible for allergy attributed to 5-CSA, by the following approximate calculation, based on QMM and linear free energy relationships principles, to estimate its potency relative to atranol. Non-chemists may prefer to skim lightly over this paragraph. Going from atranol (**7**) to 3-CSA (**6**), we exchange H ($\sigma^* = 0.49$) bonded to carbonyl for an alkyl group (for which we use a σ^* value of -0.12) bonded to carbonyl, corresponding to a difference in σ^* of -0.61 . The difference in σ^* values of the aromatic groups bonded to carbonyl, calculated as described by Perrin et al. (7), is $+0.14$ (with -0.04 as the σ value for the *ortho*-hydroxyl

and assuming the $-\text{CO}_2\text{H}$ group to be ionized). Thus, the difference in $\Sigma\sigma^*$, modelling the Schiff base reactivity of the carbonyl group, is -0.47 . Multiplying by 1.12, the $\Sigma\sigma^*$ coefficient in the published QMM for potency of Schiff base electrophiles and taking the negative logarithm, leads to an approximate estimate that 3-CSA is ~ 3.5 times less potent than atranol on a molar basis, corresponding to approximately six times less potent than atranol on a weight concentration basis. This would correspond to an LLNA EC3 (concentration required to produce a threefold increase in draining lymph node cell proliferative activity) value of $\sim 3\%$, making 3-CSA a moderate sensitizer. For aliphatic aldehydes and activated ketones, potency is also dependent on hydrophobicity, and this may or may not also be the case for *ortho*-hydroxy aromatic aldehydes and ketones. However, the difference in $\log P$, calculated with the Leo and Hansch method, between atranol and 3-CSA is not large (the positive effect of adding the C_7H_{15} alkyl group is approximately cancelled out by the negative effect of adding the ionized carboxyl group), so the estimate based on the reactivity parameter alone is adequate for present purposes.

Our overall conclusion is that 5-CSA itself is unlikely to be significantly allergenic, and is therefore unlikely to be the cause of the contact allergy reported by de Groot et al. (1). However, the isomer 3-CSA is a highly plausible contaminant of 5-CSA, and is likely to be sufficiently allergenic to account for the observed contact allergy.

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References

- de Groot A, Rustemeyer T, Hissink D, Bakker M. Contact allergy to capryloyl salicylic acid. *Contact Dermatitis* 2014; **71**: 176–190.
- Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. *J Exp Med* 1936; **64**: 643–655.
- Roberts D W, Aptula A O, Patlewicz G. Mechanistic applicability domains for non-animal based prediction of toxicological endpoints. QSAR analysis of the Schiff base applicability domain for skin sensitization. *Chem Res Toxicol* 2006; **19**: 1228–1233.
- Roberts D W, Aptula A O, Patlewicz G. Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published dataset of 106 chemicals tested in the mouse local lymph node assay. *Chem Res Toxicol* 2007; **20**: 44–60.
- Roberts D W, Aptula A O. Determinants of skin sensitization potential. *J Appl Toxicol* 2008; **28**: 377–387.
- Natsch A, Gfeller H, Haupt T, Brunner G. Chemical reactivity and skin sensitization potential for benzaldehydes: can Schiff base formation explain everything? *Chem Res Toxicol* 2012; **2**: 2203–2215.
- Perrin D D, Dempsey B, Serjeant E P. pKa Prediction for Organic Acids and Bases: London, Chapman and Hall, 1981.
- REACH Dossiers. 5-CSA (78418-01-6), 2015. Available at: <http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances#search> (last accessed 28 January 2015).
- Ezendam J, Vermeulen J P, Klerk A, Jong W H, Loveren H. A quantitative approach to assess the potency of skin sensitizers in the elicitation phase. *Toxicology* 2012; **299**: 20–24.
- Jacquet B, Leveque J L, Hocquaux M, Leger D S. Topical compositions intended for skin treatment containing salicylic acid derivatives. US Patent 4767750, 1988.
- Isaacs N. Physical Organic Chemistry, 2nd edition: Harlow, Longman, 1995.
- Kovacic P, Hillier J J Jr. The ortho/para ratio in electrophilic aromatic substitution. Mercuration and alkylation of chlorobenzene and anisole. Evidence for a coordination effect. *J Org Chem* 1965; **30**: 1581–1588.
- Ralston A W, McCorkle M R, Bauer S T. Orientation in the acylation of phenol and in the rearrangement of phenolic esters. *J Org Chem* 1940; **05**: 645–649.