Unexpected positive patch test reactions to sesquiterpene lactones in patients sensitized to the glucose sensor FreeStyle Libre

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Abstract

Background: Most diabetic patients sensitized to FreeStyle Libre react to isobornyl acrylate, with a considerable number of them also showing unexpected positive patch test reactions to sesquiterpene lactone mix (SLM) tested in the baseline series.

Objectives: To compile patch test results of subjects affected, and provide potential explanations for this association.

Patients and Methods: 53 Freestyle Libre-allergic patients were patch-tested with isobornyl acrylate and/or SLM, and several also with the components of SLM. Chromatographic analyses were performed of the glucose sensor, isobornyl acrylate, and the components of SLM.

Results: Thirty-three patients reacted positively to SLM, and 11/27 patients tested positive to alantolactone, in particular. Gas-chromatographic and spectrometric (GC-MS) analyses did not detect these chemicals in the different parts of the glucose sensor, nor in isobornyl acrylate, and the latter not detected in SLM either.

Conclusion: Significant co-sensitizations between SL on the one hand and the glucose sensor FreeStyle Libre and/or isobornyl acrylate on the other hand exist, without evidence of presence of SL via GC-MS analysis. Cross-reactions between them seem improbable. As possible hypothesis, a common precursor for both, such as camphene derivatives, may exist.

Key words: sesquiterpene lactones, FreeStyle Libre, allergic contact dermatitis, chemical analysis, GC-MS, glucose sensor, diabetes mellitus, isobornyl acrylate, medical device, simultaneous reactions.

1. Introduction:

Isobornyl acrylate (IBOA, CAS no. 5888-33-5) has recently been identified as the major culprit allergen in diabetic patients sensitized to the glucose sensor Freestyle Libre (Abbott Diabetes Care, Witney, Oxon, UK) (1), and similar medical devices (2, 3).

As previously reported (1), numerous patients allergic to Freestyle Libre also tested positive to sesquiterpene lactone mix (SLM) (Chemotechnique Diagnostics, Vellinge, Sweden) which is included in the baseline series, containing equimolar concentrations of alantolactone (0.033%, CAS no. 546-43-0), costunolide (0.033%, CAS no. 553-21-9), and dehydrocostus lactone (0.033%, CAS no. 477-43-0).

This study sought to investigate such simultaneous patch-test reactions to IBOA/FreeStyle Libre and SLM in three Contact Allergy departments in Belgium. Several hypotheses were additionally explored by means of patch tests and chemical analyses, the latter performed at the Department of Occupational and Environmental Dermatology in Malmö, in order explain such association.

2. Patients and methods

2.1 Patients

All patients included in this study suffered from diabetes mellitus type I and had presented with allergic contact dermatitis from the glucose sensor FreeStyle Libre (Abbott Diabetes Care). The results of patch tests performed between January 2016 and June 2018, in which contact allergy to this glucose sensor, IBOA, or both had been confirmed, were analyzed. Fifty-three cases from three Belgian university hospitals were involved, namely, 12, 16 and 25 patients from the Dermatology departments of Leuven (UZ Leuven), Antwerp (UZA), and Brussels (Cliniques universitaires Saint-Luc), respectively. Among the 53 patients tested, there were more females than males (33 versus 20, or 62.3% versus 37.7%, respectively), with a median age of 37 years (range: 4-76 years).

The study, as well as the data collection and analysis were conducted following protocol approval by the Institutional Ethical Committee, namely the Commission d'Ethique Biomédicale Hospitalo-Facultaire de l'Université Catholique de Louvain.

2.2. Patch tests

Fifty-three patients were included in this study, all with allergic contact dermatitis from the glucose sensor FreeStyle Libre, confirmed by a positive patch test to the adhesive part of it, to IBOA, or to both. In the respective centers patch tests were performed with the European baseline series (Chemotechnique Diagnostics and/or Allergeaze, Smartpractice, Phoenix, Arizona), including SLM 0.1% pet. and Compositae mix 2.5% pet.

Twenty-seven of the 53 patients were tested with pieces of the adhesive part of the glucose sensor FreeStyle Libre, and 52 of them with IBOA, purchased from Sigma-Aldrich (Steinheim, Germany) and diluted 0.1% in pet. by the hospital pharmacy (St Luc); an in-house preparation of IBOA 0.1% pet using the raw material obtained from Kowa Europe (Düsseldorf, Germany) was used in UZA and UZ Leuven.

With regard to the number of patients' patch tested with the individual lactones: 14 were tested with costunolide and dehydrocostus lactone, purchased from Sigma-Aldrich (Steinheim, Germany) and prepared by the hospital pharmacy (0.1% pet); 27 with alantolactone (0.1% pet)., 23 with α -methylene- γ -butyrolactone (0.01% pet.), and 25 patients with parthenolide (0.1% pet.), all obtained from Chemotechnique Diagnostics.

Patch tests were applied on the upper back and occluded for 48 hours with IQ Utra test chambers from Chemotechnique Diagnostics in Brussels, and with Allergeaze path test chambers (SmartPractice, Calgary, Canada) in Antwerp and Leuven. Patch tests were fixed with Fixomull stretch (BSN Medical, Hamburg, Germany) in Antwerp and Brussels, and with Mefix (Mölnlycke, Gôteborg, Sweden) in Leuven, respectively. Readings were performed on days (D) 2 and D4, according to the ESCD criteria (4).

2.3 Thin layer chromatography patch tests

The following extracts were prepared for thin-layer chromatography (TLC) patch testing:

- Adhesive patches removed from two FreeStyle Libre sensors were extracted in 2.5 mL ethanol for 12 hours, after which the extract was evaporated to a volume of 0.5 mL.
- The plastic covers from two sensors (with the patches removed) were cut into small pieces and extracted in 10 mL acetone for 12 hours, which was evaporated to a volume of 1 mL.
- 500 mg of a SLM petrolatum test preparation from Chemotechnique was dissolved in 5 mL heptane. The heptane solution was then extracted three times with 5 mL methanol. The methanol phases were merged and were then evaporated to a volume of approximately 2 mL. Thereafter the methanol phase was washed twice with 5 mL heptane in order to remove petrolatum residues, and evaporated to a volume of 300 µL.

In addition to the abovementioned extracts TLC patch testing was also performed with 0.1% IBOA and 0.1% costunolide solutions in acetone and ethanol, respectively.

Thin layer chromatography was performed on TLC Silica gel 60 F254 plastic sheets (Merck, Darmstadt, Germany). For each solution, several applications were made approximately 2.5 cm from each other on a line marked 2 cm from the bottom of the TLC sheet. The volumes applied on each spot were 20 µL for the extract of the adhesive patch, 30 µL for the extract of the sensor, 15 µL for IBOA, and 30 µL for the

SLM extract, and 15 µL for costunolide. The samples were eluted with a mobile phase consisting of 70% (vol/vol) heptane (Merck) and 30% (vol/vol) ethyl acetate (VWR International, Fontenay-sous-Bois, France). TLC sheets were investigated under UV light at 254 and 366 nm. The visualized spots were marked with a pencil. Thereafter the TLC sheets were cut into strips (one strip for each application) to be used for patch testing and the position of the spots were marked on the plastic backing of the TLC strips with a marker pen. One strip of each chromatogram was used as a template when reading the test, and at least one strip was left to be used for chemical investigations of areas giving positive test reactions (5). Overall, five patients were tested with TLC strips of the adhesive patch and the sensor. Only one of these patients was tested with TLC strips of IBOA, SLM extract and costunolide

2.4 Chemical analysis

Chemical analyses were performed by gas chromatography-mass spectrometry (GC-MS) at the Malmö department (1). All extracts used for TLC patch testing were analyzed as such. When analyzing the areas of the TLC strips of the adhesive patch and the sensor, the silica gel was scraped off from these areas and was then extracted in a small volume of methanol (~200µL). The extracts were then filtered and analyzed by GC-MS. In addition, analyses were also performed on an extract of circuit from a FreeStyle Libre sensor. The circuit board were cut into small pieces and were extracted in ~2 mL acetone for 4 hours. These extracts were filtered and concentrated to a volume of approximately 0.2 mL. Dilutions of IBOA in acetone and of

alantolactone, costunolide, and dehydrocostus lactone in ethanol were used as reference standards. The detection limits were estimated to 0.01 mg/mL for IBOA, 0.03 mg/mL for alantolactone, 0.3 mg/mL for costunolide and 0.1 mg/mL for dehydrocostus lactone.

3. Results

3.1 Patients and patch tests:

All 52 patients tested to IBOA were positive to it, and among 27 patients tested with the pieces of adhesive of Freestyle® Libre sensor, 22 patients reacted positively. Out of 53 patients, 33 (62.3%, 10 males and 23 females) with a mean age of 39 years, tested positively to SLM, of whom 14 were also tested to costunolide and dehydrocostus lactone, and 27 to alantolactone: three reacted positively to costunolide, one to dehydrocostus lactone, and 11 (40.7%) to alantolactone. Eleven patients (20.8%) out of 53 tested likewise presented with a positive reaction to Compositae mix, whereas only one patient reacted to parthenolide (tested in 25 cases). Only one out of the eleven patients tested with α -methylene- γ -butyrolactone reacted positively to it. All data, including patch test results and demographic data, are summarized in **Table 1**.

3.2 Thin layer chromatography patch tests

The positive reactions obtained with the TLC strips have been summarized in **Table 2** and **Fig. 1**. All 5 patients tested with the TLC strips reacted positively. Concerning the FreeStyle Libre sensor, they all reacted positively to an area that was not visible by the eye, nor observed under UV light. Concerning the more diffuse reaction in patient 5, the center of the reaction was at the same location as in the reactions presented in the

other cases. Likewise, three patients presented with a positive reaction to an area of the TLC strips of the FreeStyle Libre patch, which was not visible either. Both TLC areas had the same Rf-value ~0.6. One patient was tested with three TLCs strip containing costunolide, SLM extract, and IBOA. She reacted to one spot of the costunolide TLC and one spot of SLM TLC with the same Rf-values (~0.4). On the IBOA TLC, this patient showed a positive reaction to an area with the same Rf-value (~0.6) as the positive reactions observed for the TLC strips containing the FreeStyle Libre sensor and patch extracts.

3.3 Chemical analysis

Chromatograms of costunolide, alantolactone, dehydrocostus lactone, IBOA, FreeStyle Libre sensor extract and patch extract are shown in Online supplemental **Fig. 2**. No alantolactone, costunolide, or dehydroctostus lactone could be detected in the extracts of the different parts of sensor FreeStyle Libre. Likewise, comparing the retention time and mass spectra of GC-MS peaks did not reveal any alantolactone, costunolide or dehydrocostus lactone in IBOA (analyzed at a concentration of up to 2% in acetone) used for patch testing. Conversely, no IBOA was found in the SLM extract nor in the alantolactone, costunolide and dehydrocostus lactone solutions. All those compounds were analyzed at concentrations up to 2% in ethanol.

The TLC spots giving positive reactions for the patch as well as the sensor of Freestyle Libre were shown to contain IBOA (Online supplemental **Fig. 3**). The

positive area of the TLC of IBOA contained IBOA, and no other substance was observed in these extracts. The positive area of the costunolide TLC contained costunolide, and the positive area of the SLM extract TLC contained alantolactone, costunolide and dehydrocostus lactone, which all also had similar Rf-values when eluted individually on a TLC sheet.

4. Discussion

Sesquiterpene lactones (SLs) are a group of terpenoids present in several plant families, including Lauraceae, Magnoliaceae, and Compositae, the latter being the largest one. In most cases of allergic contact dermatitis (ACD), sensitization from SLs has been caused by cosmetics, pharmaceutical products, herbal medicines, flower handling, or consumption of SL-containing plants (6). Some SLs are characterized by an α-methylene-γ-butyrolactone ring (**Fig.** 4). Such structures, and notably, the α,β unsaturated carbonyl chemical function, prove to be good electrophiles that create a covalent binding with nucleophilic residues of skin proteins via 1,4 addition or Michael addition, responsible for their sensitizing potential (7, 8).

SLM was developed for patch testing and is estimated to detect 60 to 70% of all Compositae contact allergies (9) and is therefore included in the European baseline series (10). Interestingly, within a country, significant differences between the percentages of simultaneous reactions to SLM and Compositae mix can be observed (11). The prevalence of sensitization to SL (based on the SLM patch tests) observed in European patch-test clinics ranges from 0.1 to 2.0% (6, 12), while it was estimated to be 0.1% within the European general population (13).

Among the 53 patients tested for ACD from the glucose sensor FreeStyle Libre, IBOA, or both, more than half (62.3%) exhibited sensitization to SL, which accounts for the increased prevalence of sensitization observed in the three contact allergy units involved in this study. The sensitization prevalences to SL from 2014 to 2017 in these clinics, summarized in **Table**

3, indeed showed a relative increase in recent years. For example, at the Cliniques universitaires Saint-Luc in Brussels, the sensitization rate to SL of patients attending the contact allergy unit was 0.19% in 2015, 0.50% in 2016, and rose to 1.66% in 2017, the year in which contact-allergy problems due to glucose sensors started to appear. Over the period analyzed, from January 2016 to June 2018, 3902 patients were tested for SLM in the 3 universities. The sensitization rate to SLM during this period is 1.23%. Excluding patients tested and sensitized to IBOA, the rate decreases to 0.72%. The exact mechanism(s) of this co-sensitization remain unclear though.

4.1 Cross-reaction between SLs and IBOA

Cross-reactivity between chemical compounds has to be considered when a close relationship in sensitization frequencies is observed. IBOA along with alantolactone, costunolide and dehydrocostus, the three lactones present in the SLM, share the same chemical reactive function. Indeed, these molecules contain a carbonyl function (double bond between a carbon and an oxygen atom), and are able to induce a Michael addition; this reaction represents an addition of a nucleophile to an α, β unsaturated carbonyl compound, acting as a Michael acceptor with a skin protein, or an amino acid, responsible of the protein activity, such as cysteine (7). However, during the elicitation phase, two factors are crucial, i.e., the function of the chemical groups and also the three dimensional structure. The spatial structure of IBOA and the three SLM derivatives is completely different, hence, not likely to activate the same T-cell receptor; therefore, cross-reactivity appears quite improbable. The cross re-tests model, as proposed by Rustemeyer et al (14), a test technique that is based on

skin hyper-reactivity due to local cutaneous residual T-cells with a memory of a specific allergen, could provide evidence for distinguishing cross-reactivity from co-sensitization.

Cross-reactions between different SLs are possible by structural similarities of the α -methylene- Ɣ -butyrolactone ring (15), which, according to a study by Stampf et al, is a prerequisite for cross sensitization between them, although apparently not sufficient for sensitization. Cross reactions between alantolactone and costunolide have been described, but also between alantolactone and two types of spirolactone, containing an α -methylene- Ɣ -butyrolactone ring, which can be synthesized from camphene (15, 16). However, no cross-reactions have been observed between these lactones and α -methylene- Ɣ -butyrolactone tested separately: this molecule does not exhibit the structural homology able to activate the same lymphocytes. Similar results have been described in animal tests (17). In the present study, two patients presented co-sensitization between alantolactone and costunolide, yet only one co-reacted to both SLM and α -methylene- Ɣ -butyrolactone. Further investigations are thus necessary to investigate possible cross- reactivity between the three lactones in the SLM and IBOA, considering camphene as the starting molecule, necessary for the synthesis of these molecules.

Lastly, enzymatic, but also non-enzymatic reactions, such as autoxidation on air exposure, could result in modifications of the chemical IBOA structure that could, in turn, induce the formation of a new metabolite (18) able to cross-react with SLs.

4.2 Presence of SLs in the glucose sensor

Another potential explanation of the present surprising association is co-sensitization due to contact with SLs present in the glucose sensor. Despite our multiple and different requests, no information about the exact composition of the sensors could be obtained from the manufacturers. Analyses by GC-MS were therefore performed in order to investigate the composition of the sensor, through which the presence of IBOA was previously highlighted (1). Further analyses were performed, comparing the retention times and mass spectra of the SLM components with the different peaks observed in the chromatograms of the extracts of the different parts of the glucose sensor (*i.e.*, adhesives part, plastic part, or circuit board). However, theses analyses did not detect SLM components. However, it cannot be completely ruled out that small amounts of alantolactone, costunolide, and dehydrocostus lactone, or other SLs, are present in the sensor. The chromatographic method used is not optimal for all SLs, and costunolide gave a very broad peak, which would render its identification in small amounts in a complex mixture, such as the investigated extracts, more difficult.

Furthermore, constituents in the extracts of the FreeStyle Libre sensor and patch were separated on TLC strips. Those strips were used for patch testing in order to investigate whether the patients reacted to one or several areas on the strip. Indeed, the presence of more than one positive spot would confirm the presence of several simultaneous allergens. All patients reacted to the same area though, i.e. corresponding to IBOA in the TLC strip of the adhesive patch of FreeStyle, which was confirmed by GC-MS analysis. The results support the absence of SLM components in the sensors. Positive patch test reaction with SLM seems therefore more likely to be the expression of a cross-reaction than co-sensitization through

presence of SLs in the sensor. However, it can never be completely excluded that small amounts of SLs might be present in the sensor, but not in a sufficient quantity to cause a reaction.

The presence of plant materials in medical devices has already been reported, for example, concerning ACD caused by self-adhesive electrocardiography electrodes in a young girl (19). In this case, positive patch test reactions to Compositae mix, but not to SLM components were noted; chromatographic analysis confirmed the presence of herbal extracts in the adhesive part of the electrodes (not specified). In the case of glucose sensors, additional analyses might be required to fully eliminate the presence of related SLs or other plant components.

4.3 IBOA impurities

Foti et al reported IBOA to be an impurity of alkyl glucosides, and perhaps the sensitizing culprit in them (20). Henceforth, the presence of IBOA impurities in the SLM sample, and conversely, also of SL impurities in the IBOA sample, was explored. According to GC-MS analyses, comparing the retention times and mass spectra of both the SLM components and IBOA, the presence of the latter as an impurity could not be detected, although, again, we cannot exclude the possibility of small amounts being present. Several peaks were detected by means of GC-MS in IBOA used for patch testing and purchased from Sigma, probably corresponding to impurities. However, the retention time of these different peaks did not match with those of the SLM components, the exact nature of them not being

identified, but most appeared to have an isobornyl group in their structure. It should be noted that recently, at the Antwerp department, additional chemical analyses failed to confirm IBOA as an impurity in alkyl glucosides (21).

Sigma-Aldrich (Steinheim, Germany) purchased IBOA from Arkema (Colombes, France), who informed us that it was obtained by a chemical reaction between acrylic acid and camphene, a bicyclic monoterpene. Although the reaction product was purified via distillation, camphene residues at <1% weight could still be present. Note that camphene can also be found in SL-containing plants (22), and as an ingredient, at concentrations ranging from 0,1 to 1%, in UV curing adhesives used for medical devices, i.e., Loctite AA3926 LC MED (Henkel, Düsseldorf, Germany)(23).

4.4 Previous sensitization to SLs via antidiabetic treatments

For many years, medical research has attempted to develop new, pharmacologically active agents from natural plant sources. Several SLs have been employed in animal experiments in order to prevent or to control diabetic complications. However, the already known molecules do not contain the unsaturated methylene group, which is a prerequisite for a Michael-addition and for the allergenic properties. Patients included in the current study had not previously participated in a study protocol focusing on these drug types, whereas insulin, or other typical drugs for diabetic patients, does not contain SLs. Moreover, chemical analyses in order to formally exclude the presence of SL in the composition of the different

medications taken by diabetic patients, especially different insulin types, have not yet been undertaken.

These hypotheses must be further explored in order to better understand this surprising co-sensitization, and exclude reactions between IBOA and other constituents of the UV-curing adhesive in the sensor.

5. Conclusion

Concomitant sensitization to SLM, as present in the baseline series, has been observed in diabetes patients reacting to the glucose sensor Freestyle Libre and IBOA, or to both; however, the precise mechanism(s) remains elusive. The presence of the three components of the mix, i.e., alantolactone, costunolide, and dehydrocostus lactone within the glucose sensor, as well as impurities in the patch test materials, or in the sensor, could not be demonstrated via GC-MS analyses. Notwithstanding their very similar chemical function, cross reactions between the three sesquiterpene lactones and IBOA seem unlikely, due to their different spatial structure. Therefore, at present, co-sensitization, rather than cross-reactivity, remains the most likely explanation, and the re-testing model might enable us to effectively refute cross-reactivity. The presence of a common precursor for IBOA and lactones, such as camphene, might account for the simultaneous sensitizations observed. These hypotheses, however, should be the subject of further investigations.

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Table 1. Demographic data and patch-tests results of 53 patients positively reacting to IBOA or FreeStyle Libre.

Patient	Centre	Years	Age (years)	Sex	IBOA D2/D4	(Pieces of) adhesive of FreeStyle® D2/D4	SLM D2/D4	Compositae Mix D2/D4	Alantolactone D2/D4	Costunolide D2/D4	Dehydrocostus D2/D4	European baseline series (except SLM / compositae) D2/D4	Additional patch tests D2/D4
1	LEU	2017	59	M	+/+	NT	+/+	-/-	NT	NT	NT	-	MA series: EA -/?
2	LEU	2017	46	F	++/++	NT	+/+	-/-	NT	NT	NT	MP +/+ Ni ?+/+ Limonene -/+ Isoeugenol +/+	MA series: EA +/+
3	LEU	2017	33	M	++/+	NT	++/+	-/-	NT	NT	NT	-/-	MA series: negative
4	LEU	2017	56	F	++/+++	+/++	+/+	?/-	NT	NT	NT	MP ?+/- Ni ?+/-	MA series: EA +/++ TGDA +/++
5	LEU	2017	58	F	++/++	NT	+/+	-/-	NT	NT	NT	Colophonium +/? Ni +/+ MP +/+	MA series: negative
6	LEU	2017	42	F	-/+	NT	-/?+	-/-	NT	NT	NT	Ni +/++ Co +/+	MA series: EA -/+
7	LEU	2018	34	F	++/++	NT	+/+	?/-	NT	NT	NT	-	MA series: EA +/+
8	LEU	2017	21	F	+/+	NT	?/-	-/-	NT	NT	NT	-	MA series: EA +/+

9	LEU	2017	46	F	+/++	NT	-/?+	-/-	NT	NT	NT	MP +/+++ Ni +/++ FMI -/+	MA series: EA +/++
10	LEU	2017	62	M	+/+	NT	NT	-/-	NT	NT	NT	NT	MA series: EA ?++/?++
11	LEU	2018	42	M	++/+++	NT	+/+	+/+	NT	NT	NT	Ni -/?++ Colophonium -/+ Isopropanol -/+	MA series: EA ?++/?++
12	LEU	2018	14	F	+/++	NT	+/+	?+/+	NT	NT	NT	Quaternium 15 +/+ Cetearyl alcohol ?+/+	MA series: EA +/++
13	UZA	2016	9	M	++/++	NT	NT	NT	NT	NT	NT	NT	MA series: HPA +/+
14	UZA	2017	14	M	-/++	NT	NT	NT	NT	NT	NT	NT	
15	UZA	2017	49	F	++/++	NT	++/+++	-/+	NT	NT	NT	PPD ?+/+ Ni + /++ Neomycin - /+ FM I - /?+ MCI/MI -/+	MA series: TGDMA +++/+ BIS-GMA -/?++ HDA +/++ THFMA ++/++ TGDA +++/+++ EA -/+ HPA +/- BDA ++/++ DGDA ++ /++ TPGDA -/+

16	UZA	2017	16	F	++/++	+/+	++ /+	-/?+	NT	NT	NT	MP - / + Colophonium + /+ CAPB ?++/ - HG ?+ /- PG ?+/ - DMAPA ?+/- Limonene ?+/ - TDM -/?++	MA series: TGDMA ?+/?++
17	UZA	2017	24	F	++/++	NT	++/+	+/-?	++/++	NT	NT	MP ?+/- PG ?+/- SSO ?+/-	MA series: EA +/?++ Plants: *Chrysanthemum cinerariaefolium* ?++/?+ *Tanacetum vulgare* +/ ?
18	UZA	2017	9	F	+/+	NT	NT	NT	-/-	NT	NT	NT	MA series: DGDA -/?++ BDMA -/?++
19	UZA	2017	15	F	++/++	NT	++/++	+/+	+/-	NT	NT	Neomycin ?/- Thiuram mix +/- Ni ?++/ ?+ PG ?+ /-	MA series: BMA?++/- BDA ?++/- BA ?+/ -

20	UZA	2017	11	M	+++/+++	NT	++/++	+/+	+/+	NT	NT	Neomycin -/?+ Cr ?+/?+ MP +/+ Lanolin alcohol +/+ Mercapto mix ++/++ MCI/MI ++/++ MBT ++/ +++ Amerchol L101 +/?++ MI ++/ ++ BIT +/+ OIT ?++/?+ PG -/irr Thiuram mix - /?+ Colophonium - /?+	MA series: EA +/+ EHA +/+ BA +/?++
21	UZA	2017	11	F	+/+	NT	-/-	-/-	-/-	NT	NT	FM I +/- SSO +/-	MA series: negative
22	UZA	2018	64	M	++/++	NT	-/-	-/-	NT	NT	NT	MCI/MI -/+ Benzoic acid +/+ Bronopol -/?+ DU ?+/?+ IU ?+/- Linalool +/+ OIT ?+/?+	MA series: negative

												BIT +/+ SSO ?+/- DPG ?+/-?	
23	UZA	2018	13	M	+/+	NT	NT	NT	-/-	NT	NT	NT	MA series: negative
24	UZA	2018	64	F	+/++	NT	- /+	-/-	?+/+	NT	NT	Co +/++ Paraben mix ?/- Ni ?/?+ MCI/MI -/+	MA series: negative
25	UZA	2018	24	M	++/++	NT	+/+	-/-	+/++	NT	NT	Mercapto mix +/? Paraben mix +/irr Bronopol +/irr Parthenolide +/+ Limonene +/+ Linalool +/+	MA series: negative
[illegible]	UZA	2018	5	M	++/++	NT	-/-	-/-	+/-	NT	NT	FMI +/-	
27	UZA	2018	40	F	+/++	NT	+/+	-/-	?+/-	NT	NT	Ni -/?+ DPG +/ -	MA series: negative
28	UZA	2018	17	F	?+/++	NT	- /+	-/-	-/-	NT	NT	Ni -/?+ Limonene - /?+ CAPB ?+/+	MA series: MMA -/?+ DGDA -/?+

29	UCL	2016	9	M	NT	+/+	-/-	-/-	NT	NT	NT	-/-	MA series: Hexamethylene diisocyanate ++/++ P&G: Abitol ++/++ Hydroquinone ++/++
30	UCL	2017	12	F	+++/+++	+/+	++/++	-/-	NT	NT	NT	MP -/+ TP +/+ FMI +/+ PG +/+ BIT +/?	P&G: Epoxy resin +/+ Cycloaliphatic +/+
31	UCL	2017	41	F	++/++	+/+	-/-	-/-	NT	NT	NT	Ni ++/++	MA series: negative P&G: negative
32	UCL	2017	44	F	+++/+++	-/-	-/-	-/-	NT	NT	NT	-/-	MA series: negative P&G: negative
33	UCL	2017	52	F	++/++	++/++	+/++	-/-	NT	NT	NT	Ni ++/++	MA series: negative P&G: negative
34	UCL	2017	41	F	++/++	-/-	+/+	-/-	NT	NT	NT	FMI +/+	MA series: negative P&G: negative
35	UCL	2017	12	M	+/++	+/+	+/+	-/-	NT	NT	NT	-/-	MA series: negative

													P&G: negative
[illegible]	UCL	2017	49	M	++/+	+/+	-/-	-/-	NT	NT	NT	-/-	MA series: negative P&G: negative
37	UCL	2017	4	F	+/+	+/+	-/-	-/-	-/+	NT	NT	-/-	MA series: negative P&G: negative
38	UCL	2017	39	F	+/+	?+/-	+/+	-/-	NT	NT	NT	Co ?/+ Ni ++/++	MA series: negative P&G: negative
[illegible]	UCL	2017	65	F	+/++	+/++	+/+	-/-	-/-	NT	NT	-/-	MA series: negative P&G: negative
40	UCL	2017	13	F	+/+	-/+	-/-	-/-	-/-	-/-	+/-	-/-	MA series: HPMA -/+ plants: *Tanacetum vulgare* extract +/ -?
41	UCL	2017	26	F	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	MA series: negative P&G: negative Plants: negative

42	UCL	2018	33	M	++/++	+/++	-/+	-/-	-/-	-/-	-/-	Cr -/+	MA series: negative P&G: negative Plants: negative
43	UCL	2018	52	F	+/++	+/++	++/++	+/+	+/++	+/+	-/-	Resorcinol monobenzoate +/- Hydroquinone -/+ Propolis -/+ MP -/+	
44	UCL	2018	47	F	+/+	+/+	-/-	-/-	-/-	-/-	-/-	Colophonium -/+ PTBP-FR ++/++	MA series: BA -/+ EA +/++ HEA -/++ HDA +/++ TGDA +++/+++ TGDMA ++/++ P&G: Abitol -/+ Plants: negative
45	UCL	2018	62	F	+/+	+/+	-/-	-/-	-/-	-/-	-/-	Ni +/+ Sodium metabisulfite +/+	MA series: negative P&G: negative Plants: negative

46	UCL	2018	76	F	+/+	+/+	-/+	-/-	-/-	-/-	-/-	Co -/+ Ni +/++	MA series: negative P&G: negative Plants: negative
47	UCL	2018	42	M	+++/++	+/++	++/++	-/-	++/++	-/-	-/-	-/-	MA series: negative P&G: Abitol -/+ Plants: Alantolactone ++/++
48	UCL	2018	63	F	++/++	+/++	++/++	-/+	+/+	+/++	-/-	MP -/+	MA series: negative P&G: negative: Plants: Alantolactone +/+
[illegible]	UCL	2018	47	M	++/++	+/++	+/++	-/-	-/-	-/++	-/-	Lanolin alcohol +/+ DPG +/+ TDM -/+	P&G: Abitol -/+ Plants: negative
50	UCL	2018	51	M	+/++	-/-	-/-	-/-	-/-	-/-	-/-	Colophonium -/+ Nickel -/+ Sodium metabisulfite +/+ Limonene -:(+)	MA series: EA-/+ P&G: negative Plants : negative

51	UCL	2018	58	M	+/++	-/+	-/-	-/-	-/-	-/-	-/-	-/-	MA series: negative P&G: negative Plants : negative
[illegible]	UCL	2018	55	F	+/+	-/++	-/+	-/-	-/-	-/-	-/-	Ni -/++	MA series: negative P&G: negative Plants : negative
53	UCL	2018	66	M	++/++	-/+	-/-	-/-	-/-	-/-	-/-	-/-	MA series: negative P&G: negative Plants : negative

BA, Butyl acrylate; BDA, Butanediol diacrylate; BDMA, Butanediol dimethacrylate; BIS-GMA, Bisphenol A glycerolate dimethacrylate; BIT, benzisothiazolinone; BMA, Butyl methacrylate; CAPB, cocamidopropyl betaine; Co, Cobalt; Cr, Chromium; DGDA, Diethylene glycol diacrylate; DMAPA , Dimethylaminopropylamine; DPG, Diphenylguanidine; DU, diazolidinylurea, EA, Ethylacrylate; EHA, Ethylhexyl acrylate; F, female ; FM, fragrance mix; HDA, Hexanediol diacrylate, HEA, Hydroxyethyl acrylate; HG, Hexylene glycol, HPA, Hydoxypropyl acrylate; IU, Imidazolidinylurea; LEU, Dermatology, University Hospitals KU Leuven ; M, male ; MCI, Methylchloroisothiazolinone; MBT, Mercapto benzothiazole; MI, Methylisothiazolinone; MP, Myroxylon pereirae; MMA, Methyl methacrylate; Ni, Nickel; P&G series, plastic & glues series; NT, not tested ; PPA, *p*-Phenylenediamine; PTBP-FR, *p-tert*-butylphenol-formaldehyde [illegible], Sorbitan sesquioleate, TDM, Textile dye mix, TGDA, Triethyleneglycol diacrylate; TGDMA, Triethyleneglycol dimethacrylate; TP, tixocortol pivalate; [illegible], Tri(propylene glycol) diacrylate; THFMA, Tetrahydrofurfuryl methacrylate; UCL, Dermatology, Cliniques Universitaires Saint-Luc ; −, negative; ?+, doubtful, + to +++, positive patch test reaction

Table 2. Tests results with thin layer chromatography (TLC) strips with FreeStyle® Libre patch and sensor correlating with IBOA and SLM patch tests

Patient	Age	Sexe	TLC FreeStyle patch D2/D4	TLC FreeStyle sensor D2/D4	IBOA 0,1%	SLM
#1 (31)	45	F	-/-	area 1 +++/++(+)	+++/+++	-/-
#2 (32)	47	F	area 1 +/-	area 1 +/++(+)	+++/+++	-/-
#3 (37)	4	F	area 1 ++/++	area 1 ++/++	+/+	-/-
#4 (34)	41	F	-/-	area 1 ++/++	++/++	+/+
#5 (39)	65	F	area 1 +/+++	area 1 +/+++	+/++	+/+

Table 3. Sensitization rate of SLM contact allergy from the different Belgian universities from 2014 to 2017

University	Years	%
UZA	2014	0
	2015	0
	2016	0,43
	2017	1,02
UCL	2014	0.38
	2015	0.19
	2016	0.50
	2017	1.66
LEU	2014	0.21
	2015	0.90
	2016	0.72
	2017	0.78

UZA: University Hospital Antwerp; UCL: Cliniques universitaires Saint-Luc in Brussels; LEU: University Hospitals KU Leuven

Unexpected positive patch-test reactions to sesquiterpene lactones in patients sensitized to the glucose sensor FreeStyle Libre

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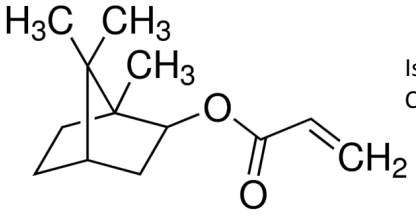
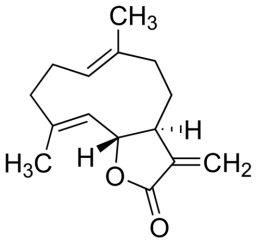
Highlights:

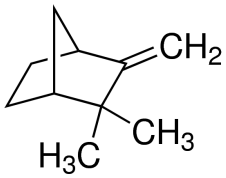
- 62,3% of diabetes patients sensitized to glucose sensor FreeStyle Libre and/or isobornyl acrylate (IBOA) were tested positively to the sesquiterpene lactone mix.
- Cross-reaction between IBOA and the three sesquiterpene lactone mix (alantolactone, costunolide, and dehydrocostus lactone) seem unlikely, due to their different spatial structure.
- Co-sensitization remains the most likely explanation notably with the presence of a common precursor for IBOA and sesquiterpene lactones, such as camphene.



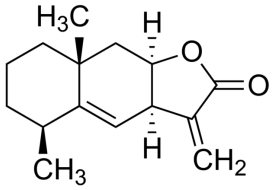
Isobornyl acrylate
CAS n° 588-33-5



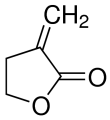
Costunolide
CAS n° 553-21-9



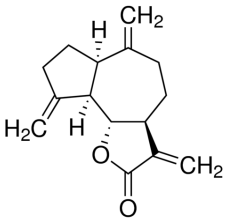
Camphene
CAS n° 5794-03-6



Alantolactone
CAS n° 546-43-0



Alpha-Methylene-
y-butyrolactone
CAS n° 547-65-9



Dehydrocostus
lactone
CAS n° 477-43-0

Fig 1: A: Day 4 reading of positive reactions observed with thin layer chromatography (TLC) strip (FreeStyle® Libre sensor) in the 5 patients tested. B: Positive reaction (D4, ++) in patient 1. C: Positive reaction (D4, +++) in patient 5.

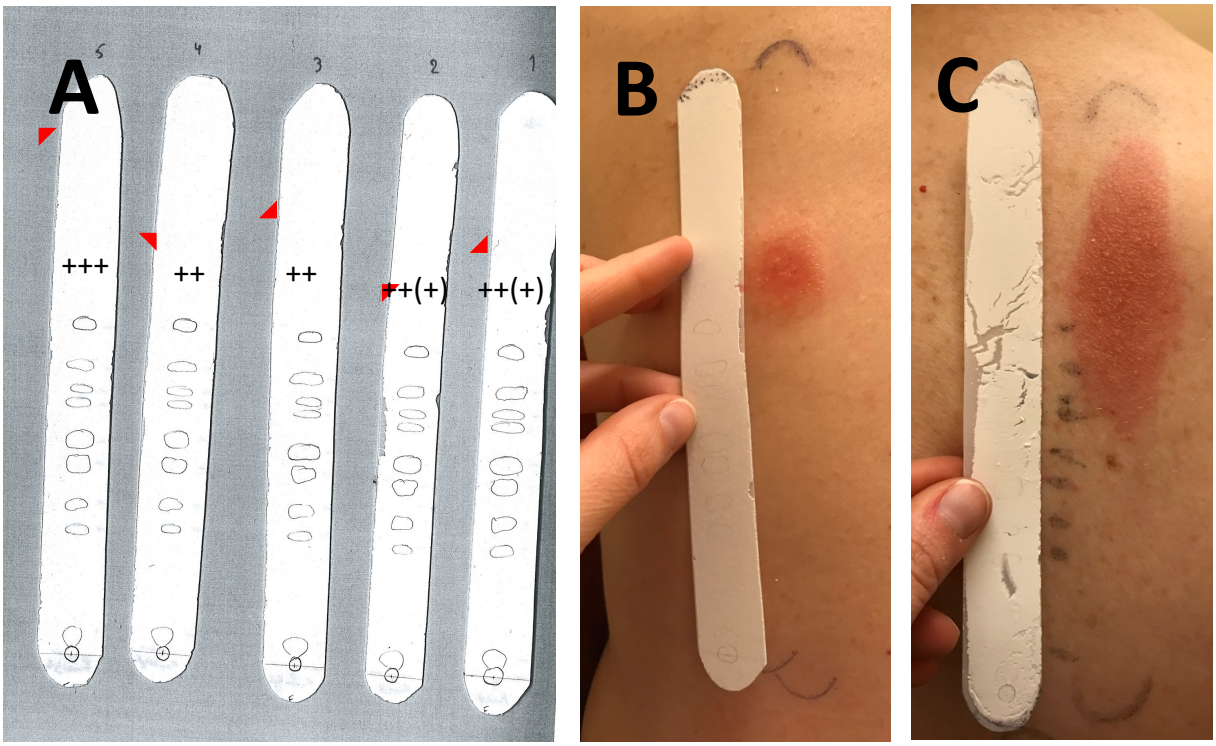


Fig 2. Total ion chromatograms of costunolide, alantolactone, dehydrocostus lactone (each 0.1% in ethanol) and isobornyl acrylate (IBOA, 0.1% in acetone) as well as separate acetone extracts of the FreeStyle Libre sensor and the adhesive patch removed from the sensor. IBOA was observed in both extracts. No traces of the sesquiterpene lactones were observed in the extracts or in the IBOA sample. No traces of IBOA were observed in the sesquiterpene lactone samples.

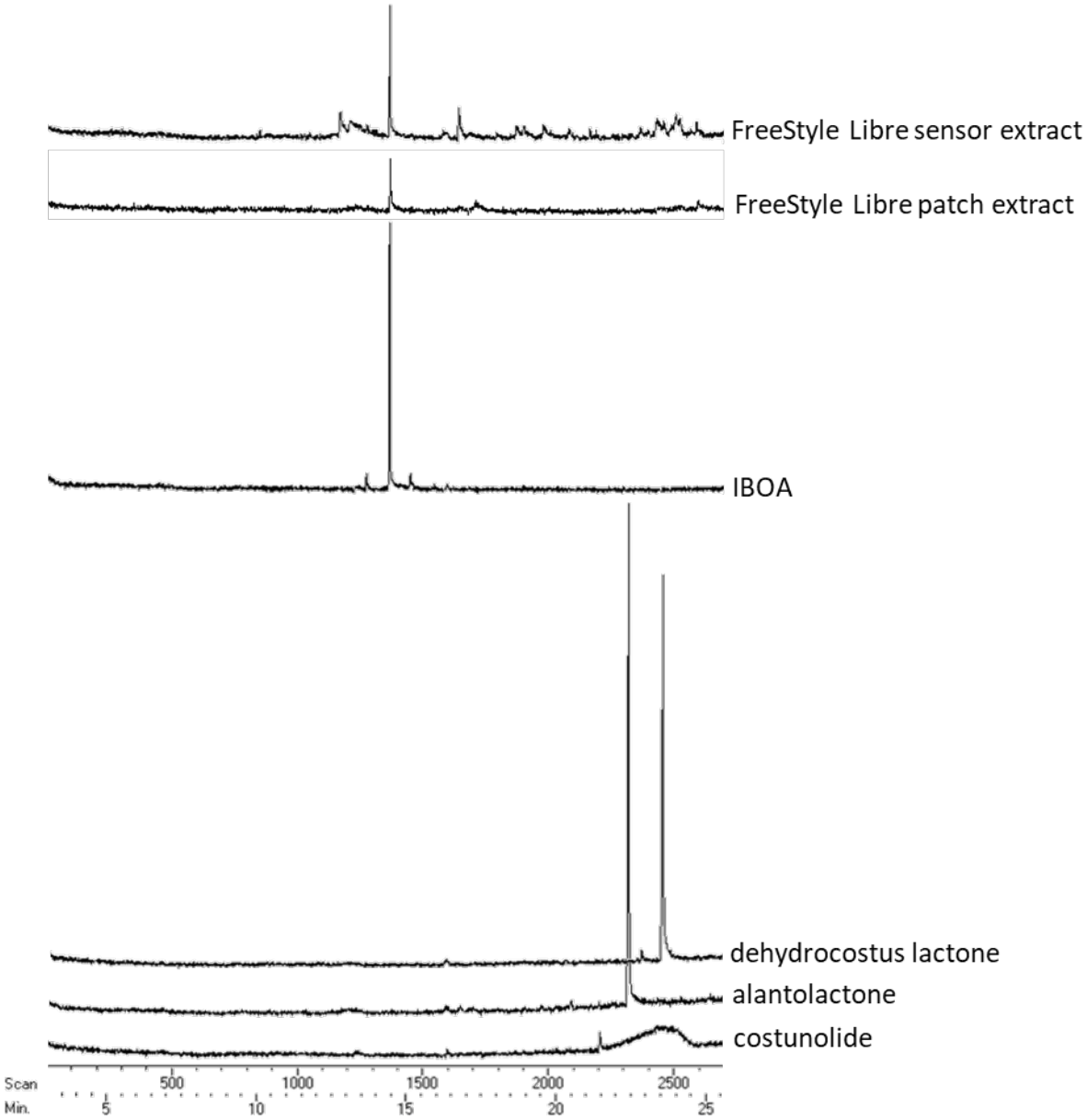


Fig. 3. a) Total ion chromatogram of an extract made from material scraped off from the area giving positive reactions in patients tested with thin-layer chromatograms of the FreeStyle Libre sensor, b) mass spectrum of the peak at 14.5 minutes, and c) mass spectrum of an isobornyl acrylate reference sample.

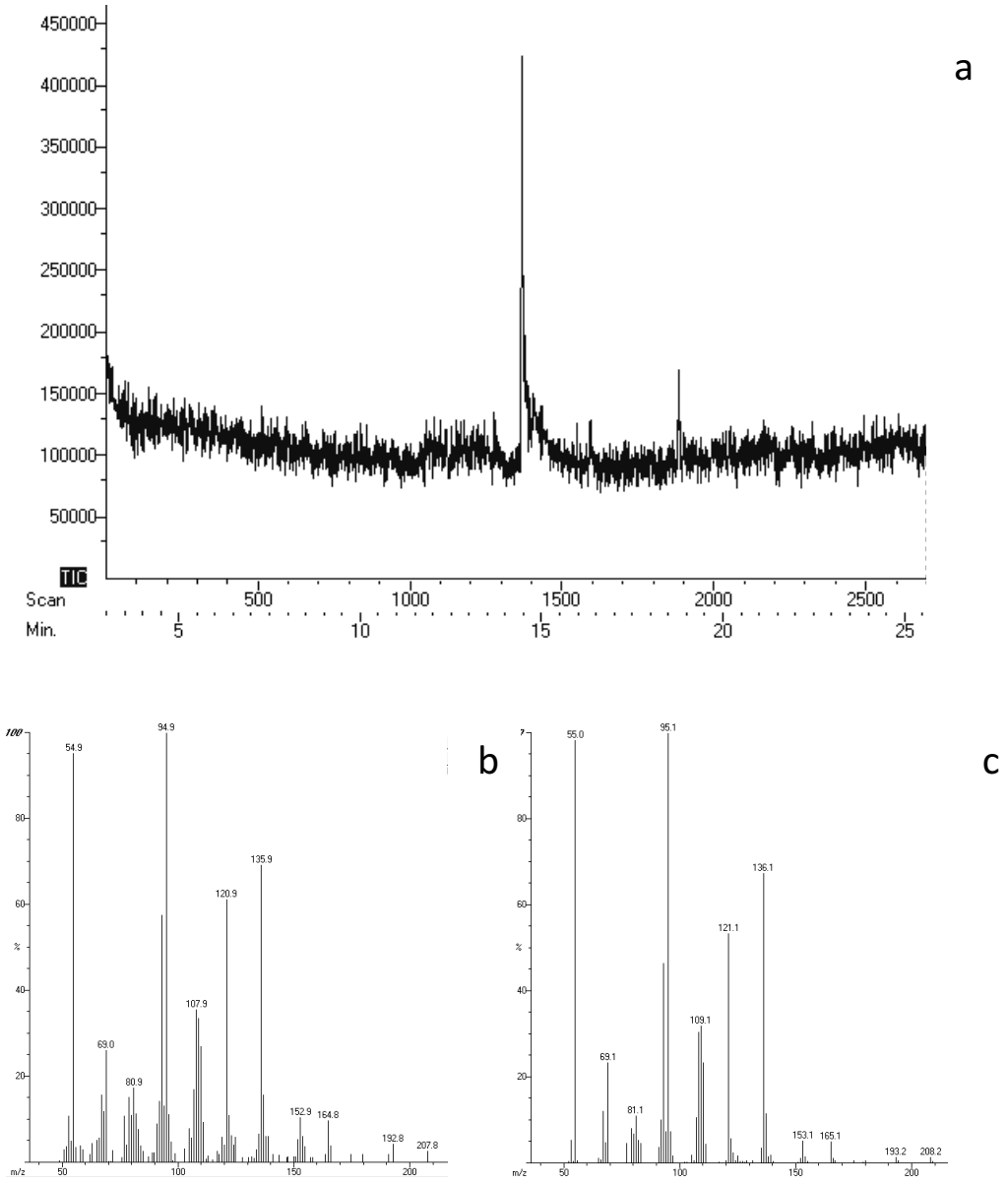
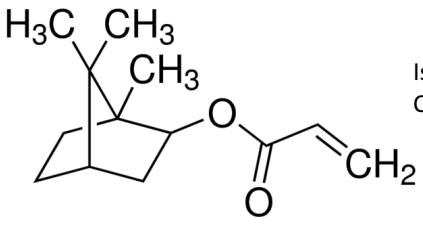
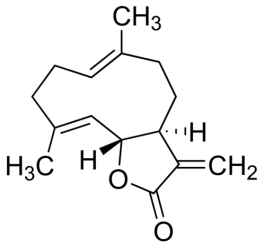


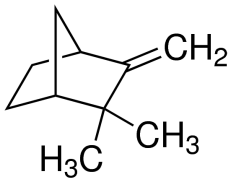
Fig 4. Molecular structures of different chemical molecules



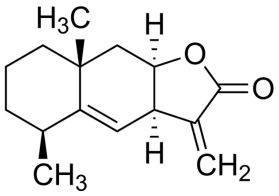
Isobornyl acrylate
CAS n° 588-33-5



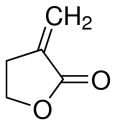
Costunolide
CAS n° 553-21-9



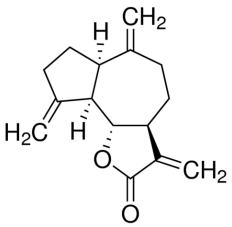
Camphene
CAS n° 5794-03-6



Alantolactone
CAS n° 546-43-0



Alpha-Methylene-
y-butyrolactone
CAS n° 547-65-9



Dehydrocostus
lactone
CAS n° 477-43-0