Double-Head Haptens. Synthesis of and Experimentally Induced Contact Sensitivity to Substances Containing Two Unrelated Haptens, Pyrocatechol and α-Methylene-γ-butyrolactone, in the Same Molecule

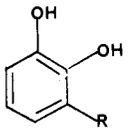
Bernard Marchand and Claude Benezra*

Laboratoire de Dermato-Chimie, Associé au CNRS (LA 31), Université Louis Pasteur, Clinique Dermatologique, CHU de Strasbourg, 67091 Strasbourg, France. *Received October 19, 1981*

A "double-head" hapten containing a pyrocatechol and an α-methylene-γ-butyrolactone moiety (3) and "monofunctional haptens" with either one of these moieties (18 and 19) connected by a six-carbon chain have been synthesized, and their sensitizing capacity was tested on guinea pigs. In the "double-head" hapten, only the pyrocatechol end is "recognized". A possible interpretation of the biological results is offered.

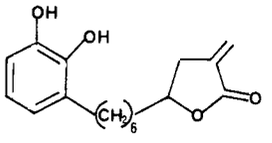
Many pharmaceutical preparations, e.g., perfumes and cosmetics, contain more than one contact sensitizer; this can result in simultaneous sensitization, a not uncommon phenomenon. This is known as "cosensitization".[1] The clinical result (sensitivity to more than one allergen or hapten) is the expression of an antigenic competition at the molecular (hapten plus carrier) and cellular (on immunologically competent cells) levels.

Because the timing of sensitization is never known precisely in Man, an experimental study in the animal (guinea pig) could provide some insight into the mechanism of cosensitization. We chose to synthesize a model compound containing two haptenic ends, one a pyrocatechol moiety and the other an α-methylene-γ-butyrolactone. The choice of these contact sensitizers (haptens) was dictated by the importance of allergic contact dermatitis (ACD) to them: 50 to 60% of the North Americans suffer from ACD to poison ivy (*Toxicodendron radicans*) or poison oak (*Toxicodendron diversilobum*);[2] the sensitizers in these plants are mixtures of penta- and heptadecylcatechols 1 and 2, respectively, called urushiols.[3]



1, R = $C_{15}H_{31}$, $C_{15}H_{29}$, $C_{15}H_{27}$, $C_{15}H_{25}$
2, R = $C_{17}H_{35}$, $C_{17}H_{33}$, $C_{17}H_{31}$, $C_{17}H_{29}$

ACD to α-methylene-γ-butyrolactones, present in a number of plants (in particular in Compositae), is very well documented.[4] This paper reports on the synthesis of "double-head" haptens 3 and on the study of their sensitizing capacity in guinea pigs.



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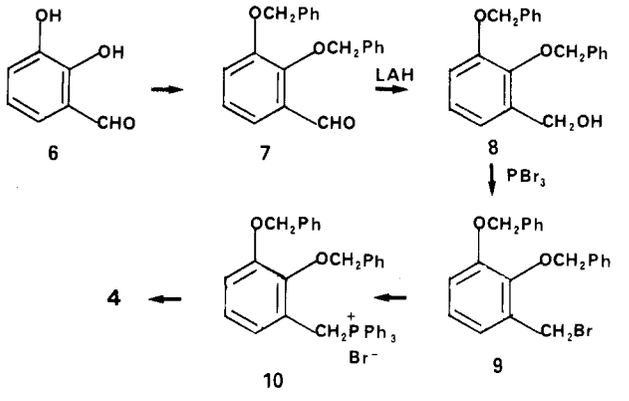
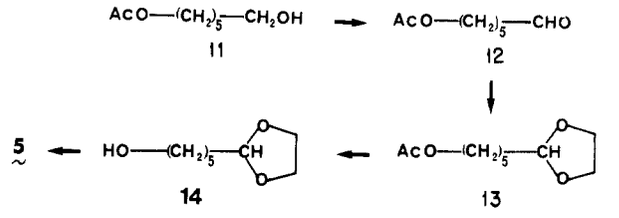
Results

Chemistry. When this work was started, it was reasoned that an appropriate synthon would be ylide 4, which could be connected to a difunctional chain such as 5, a potential starting material to make α-methylene-γ-butyrolactones by a Reformatsky reaction.[5] The Wittig

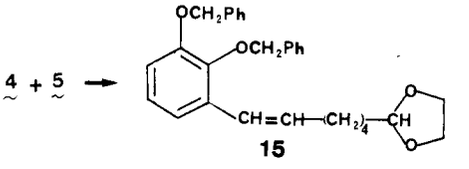
Scheme I



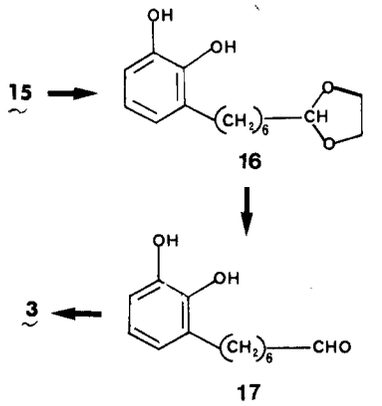
Scheme II



Scheme III

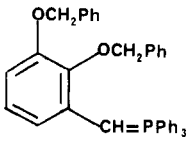


Scheme IV



(1) Rook, D. S.; Wilkinson, D. S.; Ebling, F. J. G. "Textbook of Dermatology", 3rd ed., Scientific Publications: Oxford, 1979; Vol. 1, p 372.
(2) Kligman A. E. *Arch. Dermatol.* **1958**, *77*, 149.
(3) Symes W. F.; Dawson C. R., *Nature (London)* **1953**, *171*, 841. Symes, W. F.; Dawson, C. R. *J. Am. Chem. Soc.* **1954**, *76*, 2959.
(4) Mitchell, J. C.; Dupuis, G. *Br. J. Dermatol.* **1971**, *84*, 139. Rodriguez, E.; Towers, G. H. N.; Mitchell, J. C. *Phytochemistry* **1976**, *15*, 1573.

0022-2623/82/1825-0650$01.25/0



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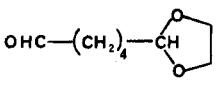

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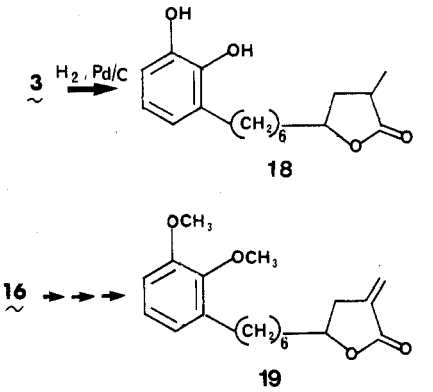
derivative 4 was prepared according to Scheme I, with an overall 63% yield from dihydroxybenzaldehyde **6**. The other synthon, **5**, was prepared from monoacetate 11[6] according to Scheme II, via oxidation, protection, ester reduction, and oxidation. The Wittig reaction of phosphorane 4 with aldehyde **5** gave a 73% yield of a 1:1 mixture of (*E*)- and (*Z*)-olefins **15** (Scheme III), as shown by NMR, a stereochemical result which can be expected from a moderately stabilized ylide,[7] such as compound 4. The last steps of the synthesis included deprotection of pyrocatechol (hydrogenation removed the benzyl group *and* the double bond to give **16**), deprotection of the aldehyde (to give **17**), and reaction with ethyl 2-(bromomethyl)acrylate[5] to give the double-head hapten **3** (Scheme IV) with a 60% overall yield from compound **15**.

All analytical data (combustion analysis and mass spectrometry) agreed with the proposed structure. The IR spectrum showed absorption at 3600, 3560, 3500–3100 (OH), and 1750 (γ-lactone) cm^{-1}. The NMR spectrum showed, inter alia, the expected signals at δ 4.55 (m, 1 H, CHO), 5.6 and 6.05 (2 t, 2 H, J = 2.6 Hz, C=CH_2), and 6.5–6.8 (m, 3 H, H aromatic).

In order for us to compare the relative sensitizing power of the two haptenic ends, *monofunctional* haptens were needed. Compound **18** was prepared by catalytic hydrogenation of the double hapten **3**. Compound **19** was prepared by methylation of compound **16**; the rest of the synthesis was as above (Scheme V).

Scheme V



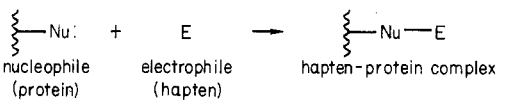
Results

Results of the elicitation tests are collected in Table I. For each group of guinea pigs is given the number of guinea pigs with 3 to 0 reactions, the average test reaction, and the total number of allergic animals. An average reaction lower than 0.5 can be considered as negative. Some preliminary comments are in order. First, all groups of guinea pigs reacted to their primary sensitizer, with reaction ranging from average (to the bihapten) to strong (to the monofunctional hapten containing the α-methylene-γ-butyrolactone group). Second, if one excludes the results of sensitization with a mixture of **18** and **19**, the only cross-reaction observable is the one of double-head hapten sensitized guinea pigs cross-reacting to the pyrocatechol monohapten. In other words, group I only "recognizes" the pyrocatechol end of the molecule. Third, group IV of guinea pigs (sensitized to the **18** plus **19** mixture) has been sensitized to *both* monofunctional haptens **18** and **19** and also to bihapten **3** (although the intensity of skin test is weak).

Discussion

Either functional group, pyrocatechol or α-methylene-γ-butyrolactone, is capable of sensitizing (cf. results of rows 2 and 3 in the table). However, when *they are located in the same molecule*, there seems to be some competition, since only the pyrocatechol end or double-head hapten is recognized. This antigenic competition is further illustrated by the results with group IV of guinea pigs. When the animals are sensitized with monofunctional lactone **18**, the skin reaction is stronger than when a mixture of monofunctional haptens (**18** and **19**) is used. Apparently, the presence of the pyrocatechol group lowers the sensitizing power of the γ-lactone. Another remarkable result is that when sensitized to the two monofunctional haptens *simultaneously*, guinea pigs are able to cross-react to the double head hapten.

In the accepted mechanism of ACD,[8] it is generally believed that in order to become antigenic, a hapten must bind covalently to a carrier, presumably a protein. Since proteins are rich in nucleophilic groups (such as NH_2, SH, and imidazole), they probably attack an electrophilic hapten, forming a covalent bond.



Although the α-methylene-γ-butyrolactone is clearly a good Michael acceptor,[9] pyrocatechol must first oxidize into *o*-quinone to acquire electrophilic properties.[10] The nature of the carrier is not known. It could, for instance, be a surface protein in epidermal cell membranes, perhaps Langerhans cells whose immunological properties are more and more recognized.[11]

Liberato et al.[12] have shown recently that pentadecylcatechols were capable of undergoing nucleophilic addition with model sulfhydryl or amino compounds, while we[9] and others[13] have shown that covalent bonds were formed when α-methylene-γ-butyrolactones were reacted with amino acids or proteins. Therefore, two covalent bond complexes can form with the double-head hapten **3**, while only one covalent bond complex would form with monofunctional haptens **18** or **19**.

In recent papers,[14] it was suggested that the pyrocatechol chain could become "anchored" to the cell membrane

(5) Löffler, A.; Pratt, R. D.; Pucknat, J.; Gelbard, G.; Dreiding, A. S. *Chimia* **1969**, *23*, 413. Reininger, K.; Schmidt, U. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 457.
(6) Babler, J. H.; Coghlan, M. J. *Tetrahedron Lett.* **1979**, *22*, 1971.
(7) Schlosser, M. *Top. Stereochem.* **1970**, *5*, 4–13.
(8) Polak, L. "Immunological Aspects of Contact Sensitivity; an Experimental Approach"; Karger: Basel, 1980.
(9) Dupuis, G.; Benezra, C.; Schlewer, G.; Stampf, J. L. *Mol. Immunol.* **1980**, *17*, 1045.
(10) Byck, J. S.; Dawson, C. R. *Anal. Biochem.* **1968**, *25*, 123.
(11) Stingl, G. *Int. J. Dermatol.* **1980**, *19*, 189. Shelley, W. B.; Juhlin, L. *Nature (London)* **1976**, *261*, 46.
(12) Liberato, D.; Byers, V. S.; Dennick, R. G.; Castagnoli, N., Jr. *J. Med. Chem.* **1981**, *24*, 28.
(13) Kupchan, S. M.; Fessler, D. G.; Eakin, M. A.; Giacobbe, T. J. *Science* **1970**, *168*, 376. Dupuis, G.; Mitchell, J. C.; Towers, G. H. N. *Can. J. Biochem.* **1974**, *52*, 575.
(14) Byers, V. S.; Castagnoli, N., Jr.; Epstein, W. L. *J. Clin. Invest.* **1979**, *64*, 1449. Byers, V. S.; Epstein, W. L.; Castagnoli, N., Jr.; Baer, H. *Ibid.* **1979**, *64*, 1437.

Table I. Results of Open Epicutaneous Tests on Sensitized Guinea Pigs

tested[a] to:	3 (Pyr-Lact)[b]		18 (Pyr-)[b]		19 (-Lact)[b]		controls[e]	
sensitized[d] to	av skin reaction[c]	no. of sensitive animals	av skin reaction	no. of sensitive animals	av skin reaction	no. of sensitive animals	av skin reaction	no. of sensitive animals
3 (Pyr-Lact)	0.9	6/8	0.8	6/8	0	0/8	0	0/4
18 (Pyr-)	0.2	3/8	1.4	7/8	0	0/8	0	0/4
19 (-Lact)	<0.1	1/8	0	0/8	2.6	8/8	0	0/4
18 + 19 (1:1 mixture)	0.9	8/8	1.1	8/8	1.1	8/8	0	0/4

[a] Tests for the double-hapten 3 were performed in 2% ethanol-CH_2Cl_2 (1:1) solutions; this is a 0.072 M solution. All other compounds were tested at the same molar concentration. The solution (0.025 mL) was deposited on a circular 2-cm² area of the animal shaved flank. [b] Pyr stands for unprotected pyrocatechol, Lact for α-methylene-γ-butyrolactone; thus, Pyr-Lact is a double-head hapten, while Pyr- and -Lact are monohaptens. [c] This number refers to the average skin reaction: 0 = no reaction, 1 = erythema on the test area, 2 = erythema and swelling in the test area, 3 = erythema plus swelling going well beyond the test area. [d] Sensitization was effected according to the Freund complete adjuvant technique (FCAT):[8] stable emulsion of the hapten (5%) in a 1:1 FCA-saline mixture was injected intradermally in the shaved nuchal region of the animal; this injection was repeated 4 times on alternate days. After 15 days rest, the animal was tested (see footnote a). [e] Controls were injected intradermally (on alternate days, five injections in all) with a 1:1 FCA-saline emulsion.

through hydrophobic interaction. One can imagine that such a mechanism could be operating here and, therefore, that a covalent bond formation between the hapten and a carrier would not be necessary for inducing or eliciting ACD.

In order to decide between the two hypotheses (covalent hapten–carrier bond formation or "anchorage"), further experiments, such as radiolabeling of compounds **3**, **18**, and **19**, are needed and are in progress in our laboratory. The results will be reported elsewhere.

Experimental Section

Melting points were observed on a 510 Büchi Tottoli apparatus and are uncorrected. Infrared spectra were recorded on a Beckman Acculab 1 spectrophotomer, and absorptions are expressed in reciprocal centimeters. ^1H NMR spectra were recorded on a 24B Perkin-Elmer Hitachi NMR spectrometer (60 MHz); chemical shifts are reported in δ values (parts per million) relative to Me_4Si (δ 0.0) and coupling constants are in hertz (s = singlet, d = doublet, t = triplet, q = quartet, br = broad). The combustion analyses were effected by the Centre de Microanalyse du CNRS. By usual workup is meant extraction with a solvent (methylene chloride or ethyl ether), washings with water, 5% aqueous $NaHCO_3$, or HCl, drying over $MgSO_4$, and removal of solvent. Analytical thin-layer chromatography (TLC) was conducted on precoated TLC plates, silica gel 60 F 254, layer thickness 0.25 mm, from Merck, Darmstadt. Silica gel column for chromatography utilized Merck silica gel 60, 70–230 mesh ASTM. The following Abbreviations are used: EE, ethyl ether; PE, petroleum ether; Hex, hexane; THF, tetrahydrofuran; Me_2SO, dimethyl sulfoxide: LAH, $LiAlH_4$; rt, room temperature.

2,3-Bis(benzyloxy)benzaldehyde (7). This compound was prepared using a slight modification (Me_2SO instead of EtOH as the solvent) of a described[15] procedure from 2,3-dihydroxybenzaldehyde (10.0 g, 72.4 mmol). The product obtained (19.3 g, 77% yield) was crystalline: mp 90 °C (lit.[15] mp 90 °C); IR 1680, 1600, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.2 (br s, 4 H, OCH_2Ph), 7–7.4 (m, 13 H, Ar H), 10.2 (s, 1 H, CHO).

2,3-Bis(benzyloxy)benzyl Alcohol (8). Into a three-necked flask equipped with a reflux condenser, an argon inlet, and an addition funnel containing LAH (0.46 g, 12.5 mmol) and THF (freshly distilled from LAH), under vigorous stirring, a solution of aldehyde **7** (15.6 g, 49.0 mmol) in THF (40 mL) was added dropwise. After the addition was completed, the mixture was stirred for 1 h, and excess LAH was hydrolyzed with AcOEt; a small amount of dilute HCl was then added to dissolve the aluminum salts. After usual workup and recrystallization from E/PE, 15.0 g (95.6%) of white crystals was obtained: mp 100 °C (lit.[15] mp 100 °C); IR ($CHCl_3$) 3600, 3400, 1600, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.8 (br s, 1 H, OH), 4.5 (s, 2 H, CH_2OH), 5.05 (s, 2 H, CH_2Ph), 5.1 (s, 2 H, CH_2Ph), 6.9 [br s, 3 H, $C_6H_3(OCH_2Ph)_2$], 7.2–7.5 (m, 10 H, $CH_2C_6H_5$).

2,3-Bis(benzyloxy)benzyl Bromide (9). A three-necked flask equipped with a condenser, an argon inlet, and an addition funnel and containing a solution of **8** (16.5 g, 51.6 mmol) in freshly distilled THF (50 mL) was cooled in an ice bath. Then, freshly distilled PBr_3 (6 mL) in THF (40 mL) was added dropwise for 1 h. After stirring for 6 h, the mixture was hydrolyzed by cautiously adding $NaHCO_3$ (50 mL of 5% aqueous solution) and extracted as usual. The residue was purified by column chromatography (elution with Hex/EE, 75:25), yielding 17.1 g (87%) of white crystals: mp 94–95 °C; IR ($CHCl_3$) 1600, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.5 (s, 2 H, CH_2Br), 5.1 (s, 2 H, CH_2Ph), 5.15 (s, 2 H, CH_2Ph), 6.95 [s, 3 H, $C_6H_3(CH_2Ph)_2$], 7.35 [br s, 10 H, $CH_2C_6H_5$). Anal. ($C_{21}H_{19}BrO_2$) C, H, Br.

[2,3-Bis(benzyloxy)benzyl]triphenylphosphonium Bromide (10). To compound **9** (17.1 g, 44.6 mmol) dissolved in dry THF (50 mL) was added PPh_3 (11.7 g, 44.6 mmol) in one portion. After the mixture was stirred overnight, filtration gave 28.2 g (98%) of **10** as white crystals, mp 141 °C. Anal. ($C_{39}H_{34}BrO_2P$) C, H, Br, P.

6-Acetoxyhexanol This compound was prepared from 1,6-hexanediol (100.0 g, 847 mmol) using a procedure described by Babler;[6] 81.0 g (506 mmol, 60% yield) of compound **11** was obtained after column chromatography (elution with EE/Hex, 1:1), as an oil; IR ($CHCl_3$) 3600, 3600–3200, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.1–1.9 [m, 8 H, $(CH_2)_4$], 2.0 (s, 3 H, $OCOCH_3$), 3.4 (s, 1 H, OH), 3.6 (t, 2 H, J = 6 Hz, CH_2OH), 4.0 (t, 2 H, J = 6 Hz, CH_2OAc). Anal. ($C_8H_{14}O_3$) C, H.

6-Acetoxy-1,1-(ethylenedioxy)hexane (13). 6-Acetoxyhexanal (**12**) was prepared by Collins oxidation[16] of compound **11** (10.0 g, 62.5 mmol): yield 7.0 g (44.3 mmol, 71%) as an oil; IR ($CHCl_3$) 2720, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5 [br s, 8 H, $(CH_2)_4$], 2.0 (s, 3 H, $OCOCH_3$), 4.0 (t, 2 H, J = 6 Hz, CH_2OAc), 9.8 (t, 1 H, J = 1.5 Hz, CHO).

The compound is protected immediately following isolation, as follows. In a flask equipped with a Dean–Stark apparatus and a reflux condenser were added compound **12** (7.0 g, 44.3 mmol), *p*-TsOH (0.10 g), ethylene glycol (8.24 g, 132.9 mmol), and toluene (120 mL). The mixture was refluxed overnight; after evaporating the toluene, the resulting oil was worked up as usual. The residue was purified by column chromatography (elution with EE/Hex, 1:1), giving **13** (8.66 g, 97%) as an oil: IR ($CHCl_3$) 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5 [br s, 8 H, $(CH_2)_4$], 2.0 (s, 3 H, $OCOCH_3$), 3.9 (m, 4 H, OCH_2CH_2O), 4.0 (t, 2 H, J = 4 Hz, CH_2OAc), 4.8 (t, 1 H, J = 3.6 Hz, $HCOCH_2CH_2O$). Anal. ($C_{10}H_{18}O_4$) C, H.

1,1-(Ethylenedioxy)-6-hydroxyhexane (14). Compound **13** (7.0 g, 34.6 mmol) was treated with LAH (0.69 g, 18.2 mmol) in THF (50 mL) as above. After usual workup, compound **14** (3.8 g, 74% yield) was obtained as an oil: IR ($CHCl_3$) 3640, 3600–3100; ^1H NMR ($CDCl_3$) δ 1.4 [br s, 8 H, $(CH_2)_4$], 3.5 (t, 2 H, J = 5.3

(15) Merz, K. W.; Fink, J. *Arch. Pharm.* **1956**, *289*, 347.

(16) Ratcliffe, R.; Rodehorst, R. *J. Org. Chem.* **1970**, *35*, 4000.

Hz, CH_2OH), 3.8 (m, 4 H, OCH_2CH_2O), 4.7 (t, 1 H, J = 3.6 Hz, $HCOCH_2CH_2O$). Anal. ($C_8H_{16}O_3$) C, H.

1-[2,3-Bis(benzyloxy)phenyl]-7,7-(ethylenedioxy)-1-heptene (15). Compound 14 (3.9 g, 26.35 mmol) was oxidized by Collins method as described above.[16] After column chromatography (elution with EE/Hex, 1:1), compound 5 (1.2 g, 7.6 mmol, 31%) was obtained as an oil: IR ($CHCl_3$) 2700, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5 [m, 8 H, $(CH_2)_4$], 2.4 (br t, 2 H, J = 5.6 Hz, CH_2CHO), 3.8 (m, 4 H, OCH_2CH_2O), 4.7 (t, 1 H, J = 3.6 Hz, $HCOCH_2CH_2O$), 9.6 (t, 1 H, J = 1.3 Hz, CHO). This compound was directly used in the Wittig reaction as follows. In a three-necked flask equipped with a reflux condenser, an argon inlet, and a serum cap was added a suspension of 10 (6.45 g, 10.0 mmol) in dry THF (20 mL). Through the serum cap by means of a syringe was then added butyllithium (5.0 mL, 2 N). When all the BuLi was added, the resulting solution was red and clear. After the solution was stirred for 0.5 h at room temperature, compound 5 (1.58 g, 10.0 mmol) was added. After stirring for another hour, the mixture was refluxed 3 h and then cooled and worked up as usual. The residue was purified by column chromatography (elution with Hex/EE, 75:25) to give compound 15 (3.12 g, 73% yield) as an oil: IR ($CHCl_3$) 3000, 2920, 2840, 1590, 1570; ^1H NMR ($CDCl_3$) δ 1.5 [br s, 6 H, $(CH_2)_3$], 2–2.5 (m, 2 H, $CH{=}CHCH_2$), 3.8 (m, 4 H, OCH_2CH_2O), 4.8 (t, 1 H, J = 3.6 Hz, $HCOCH_2CH_2O$), 4.9 (s, 2 H, CH_2Ph), 5.0 (s, 2 H, CH_2Ph), 5.7 (dt, 1 H, $CH_B{=}CH_A$–Ar, J_{AB} = 11.6 Hz, J_{BX} = 7.0 Hz, Z isomer), 6.3 (dt, 1 H, $CH_B{=}CH_A$–Ar, J_{AB} = 15.3 Hz, J_{BX} = 7.0 Hz, E isomer), 6.6 (dt, 1 H, CH_B–CH_A–Ar, J_{AB} = 15.3 Hz, J_{AX} = 2 Hz, E isomer), 6.7 (d, 1 H, CH_B–CH_A–Ar, J_{AB} = 11.6 Hz, Z isomer), 6.85 [m, 3 H, $C_6H_3(OCH_2Ph)_2$], 7.3 (m, 10 H, $CH_2C_6H_5$). Anal. ($C_{29}H_{32}O_4$) C, H.

1-(2,3-Dihydroxyphenyl)-7,7-(ethylenedioxy)heptane (16). Compound 15 (3.0 g, 6.7 mmol) was dissolved in a mixture of AcOEt (10 mL) and EtOH (20 mL) and hydrogenated in the presence of a Pd/charcoal catalyst (0.050 g) in a Parr apparatus under a hydrogen pressure of 50 psi for 15 h at room temperature. After the solvent was removed, the crude was purified by recrystallization from Hex/EE to give compound 16 (1.65 g, 93% yield) as white crystals: mp 67 °C; IR ($CHCl_3$) 3600, 3540, 3500–3200, 1610, 1590 cm^{-1}; ^1H NMR (acetone-d_6) δ 1.4 [br s, 10 H, $(CH_2)_5$], 2.6 (t, 2 H, J = 6.6 Hz, Ar CH_2), 3.8 (m, 4 H, OCH_2CH_2O), 4.8 (t, 1 H, J = 3.6 Hz, $HCOCH_2CH_2O$), 5.8–6.8 (m, 2 H, OH), 6.6 [br s, 3 H, $C_6H_3(OH)_2$]. Anal. ($C_{15}H_{22}O_4$) C, H.

5-[6-(2,3-Dihydroxyphenyl)hexyl]-3-methylene-2-dihydrofuranone (3). Compound 16 (0.500 g, 1.88 mmol) and p-TsOH (0.020 g) in dry acetone (20 mL) were refluxed under argon for 2 h. After the acetone was removed, the residue was dissolved in CH_2Cl_2 (50 mL) and washed with water. After the solution was dried and the solvent was removed, the crude product was shown by ^1H NMR to be a mixture of aldehyde 17 and ketal 16 in a 2:1 ratio (both compounds have the same R_f in TLC). Without further purification, the crude product was dissolved in dry THF (10 mL) in a flask fitted with a condenser and an argon inlet. After 0.097 g of freshly prepared Zn powder was added, the mixture was heated to 60–70 °C, and methyl 2-(bromomethyl)acrylate[5] (0.266 g, 1.37 mmol) was added through the reflux condenser. After the addition was completed, the mixture was refluxed for 3 h and then cooled to room temperature. The residue was removed by filtration, the filtrate was added to hydrochloric acid (~1 N, 10 mL), and the solution was stirred for a few minutes. After extraction with CH_2Cl_2 (3 × 50 mL) and removal of the solvent, the crude product was purified by column chromatography (elution with Hex/EE, 75:25) to give 0.250 mg of 3 and 0.150 mg of ketal 16. The yield from reacted ketal was 65%: white crystals; mp 78 °C; IR ($CHCl_3$) 3600, 3560, 3500–3100, 2940, 2860, 1750, 1660, 1620, 1590 cm^{-1}; ^1H NMR (acetone-d_6) δ 1.2–2.4 [m, 10 H, $(CH_2)_5$], 2.65 (t, 2 H, J = 7.8 Hz, Ar CH_2), 3.0 (m, 2 H, $CH_2C{=}C$), 4.55 (m, 1 H, CHO), 5.6 (t, 1 H, J = 2.6 Hz, C=CH), 6.05 (t, 1 H, J = 2.6 Hz, C=CH), 6.5–6.8 [m, 3 H, $C_6H_3(OH)_2$], 7.7 (m, 2 H, OH). Anal. ($C_{17}H_{22}O_4$) C, H.

1-(2,3-Dimethoxyphenyl)-7,7-(ethylenedioxy)heptane (20). Compound 16 (0.550 mg, 2.07 mmol) was dissolved in acetone (10 mL), and Na_2CO_3 (0.2 g) and methyl iodide in excess (1 mL, 16.06 mmol) were added. After refluxing for 4 h, the mixture was worked up as usual and purified by column chromatography (elution Hex/EE, 1:1) to give 0.590 g (97%) of an oil: IR ($CHCl_3$) 3000, 2930, 2840, 1590, 1570 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5 [m, 10 H, $(CH_2)_5$], 2.65 (t, 2 H, J = 7.3 Hz, Ar CH_2), 3.85 (br s, 6 H, OCH_3), 4.85 (t, 1 H, J = 3.5 Hz, $HCOCH_2CH_2O$), 6.8 [m, 3 H, $C_6H_3(OCH_3)_2$]. Anal. ($C_{17}H_{26}O_4$): C, H.

5-[6-(2,3-Dimethoxyphenyl)hexyl]-3-methylene-2-dihydrofuranone 19 was prepared as above: yield 52% (from reacted ketal 20); oil; IR ($CHCl_3$) 3000, 2940, 2850, 1760, 1660, 1590, 1570 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4 [m, 10 H, $(CH_2)_5$], 2.6 (t, 2 H, J = 8 Hz, Ar CH_2), 3.0 (m, 2 H, $CH_2C{=}C$), 3.8 (br s, 6 H, OCH_3), 4.5 (m, 1 H, CHO), 5.55 (t, 1 H, J = 2.7 Hz, C=CH), 6.15 (t, 1 H, J = 2.7 Hz, C=CH), 6.1–7.0 [m, 3 H, $C_6H_3(OCH_3)_2$]. Anal. ($C_{19}H_{26}O_4$) C, H.

5-[6-(2,3-Dihydroxyphenyl)hexyl]-3-methyl-2-dihydrofuranone (18). Compound 3 (0.300 g, 1.02 mmol) was dissolved in EtOH (10 mL) and hydrogenated in the presence of a Pd/charcoal catalyst (0.010 g) as described above. After the solvent was removed, the crude product was purified by recrystallization from EE/Hex to give compound 18 (0.235 g, 78%) as white crystals: mp 92 °C; IR ($CHCl_3$) 3600, 3540, 3500–3100, 2920, 2840, 1760, 1610, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4 (m, 16 H), 2.6 (m, 2 H, Ar CH_2), 4.1–4.5 (m, 1 H, CHO), 5.1–5.8 (m, 2 H, OH), 6.65 [br s, 3 H, $C_6H_3(CH)_2$]. Anal. ($C_{17}H_{24}O_9$) C, H.

Biological Assays. Albino Himalayan spotted Füllingsdorf (from Hoffmann-La Roche, Basel) guinea pigs weighing from 300 to 500 g were sensitized as described by Klečak:[17] on alternate days, the hapten, emulsified in Freund's complete adjuvant (FCA), was injected intradermally (0.1 mL) in the shaved nuchal region of the animal (in all, five injections). The following sensitizing emulsions were used: bihapten 3, 5% w/v in a 1:1 FCA–saline emulsion; monohapten 18, 5% w/v in a 1:1 FCA–saline emulsion; monohapten 19, 5% w/v in a 1:1 FCA–saline emulsion; and a 1:1 mixture (w/w) of haptens 18 and 19, 10% w/v in all, in a 1:1 FCA–saline emulsion.

After 15 days rest, the elicitation was conducted by an open epicutaneous test (OET): 25 μL of a 2% solution of the bihapten 3 (0.072 M) in ethanol–CH_2Cl_2 (1:1) was deposited on the shaved flank of the animal (on a 2-cm^2 surface by a standard circular stamp). All other solutions were 0.072 M. Tests were read at the 24th h using the following scale: 0 = no reaction; 0.5 = slight erythema not covering the whole test area; 1 = erythema covering all the test area; 2 = erythema plus swelling of the test area; 3 = erythema plus swelling going well beyond the test area.

Before any sensitization, irritation thresholds (primary toxicity) were determined on FCA-injected controls (same procedure as above for elicitation). All the compounds were nonirritating at the 2% concentration.

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