l-*N*,*N*-**Dimethyl-2-carboxypiperidinium Chloride (7).** Compound **7** was prepared by the procedure described above in 76% yield; mp 223.5 °C (lit.[17] mp 224–225 °C).

(±)-*N*,*N*-**Dimethyl-3-carboxypiperidinium Chloride (8).** Compound **8** was prepared by the procedure described above in 84% yield; mp 287–288 °C (lit.[17] mp 285–287 °C).

(±)-3-(Palmitoyloxy)-4-aminobutyric Acid Hydrochloride (10). A 1.0-g sample (0.0084 mol) of 3-hydroxy-4-aminobutyric acid was dissolved in 10 mL of trifluoroacetic acid and warmed with stirring to 60 °C on a water bath. Palmitoyl chloride (7.33 g, 0.0252 mol) was slowly added and the reaction allowed to stir for 6 h. A 2.0-mL portion of methanol was then added to quench the reaction, the mixture was cooled, and 100 mL of acetone was added. The mixture was refrigerated overnight to effect crystallization, and the product was collected by filtration and washed with hexane. The crude solid was purified on a cation-exchange column as previously described. Recrystallization from methanol/ether yielded **10** as a white solid: 2.66 g (80.4%); mp 178–180 °C. Anal. ($C_{20}H_{40}NO_4Cl$) C, H, N.

l-*N*,*N*-**Dimethyl-*trans*-2-carboxy-4-(palmitoyloxy)-pyrrolidinium Chloride (11).** Compound **11** was prepared by the procedure described for compound **10** and was isolated as a white solid in 94% yield: mp 107–108 °C. Anal. ($C_{23}H_{44}NO_4Cl$) C, H, N.

Cyclohexene-1-carboxylic Acid (12). Compound **12** was prepared by the method of Moriconi and Mazzocchi[13] in 68% overall yield; bp 91–93 °C (0.25 mm) (lit.[13] bp 97–99 °C (1.0 mm)).

***trans*-2-(Methylamino)cyclohexanecarboxylic Acid Hydrochloride (13).** A 5.0-g sample (0.04 mol) of compound **12** and 30 mL (corresponding to 0.386 mol) of 40% aqueous methylamine were sealed in a steel bomb and heated at 140–160 °C for 48 h. The solvent was removed from the reaction mixture under reduced pressure, and the residue was dissolved in 50 mL of 1.0 N aqueous hydrochloric acid and extracted with three 50-mL portions of ether. The aqueous layer was then evaporated, and the residue was taken up in 20 mL of water and applied to a cation-exchange column as previously described. The column was eluted with 500 mL of 1.5 N aqueous HCl and the eluate evaporated to yield **13** as a viscous oil. Further purification was accomplished on a silica gel column (1.5 × 75 cm, eluted with chloroform/methanol/acetic acid (60:40:5). Repeated attempts at crystallization were unsuccessful; yield 3.21 g (51%). Anal. ($C_8H_{16}NO_2Cl$) C, H, N.

***trans*-2-(Trimethylammonio)cyclohexanecarboxylic Acid Chloride (14).** A 3.0-g portion of compound **13** (0.0155 mol) and 5.21 g of sodium bicarbonate (0.062 mol) were dissolved in previously dried methanol, and to this mixture was added 6.6 g (0.0465 mol, 2.9 mL) of methyl iodide. The reaction mixture was allowed to reflux for 72 h, with an additional 2.2 g (0.0155 mol, 0.97 mL) of methyl iodide being added every 24 h. The solvent was removed in vacuo, and the residue was dissolved in water and applied to a cation-exchange column as previously described. The eluate was evaporated and the product was separated from the residual salt by taking it up several times in dry methanol. Purification on silica gel as previously described and recrystallization from methanol/ether gave pure **14** as colorless needles: 1.28 g (37.2%); mp 257–258.5 °C. Anal. ($C_{10}H_{20}NO_2Cl$) C, H, N.

Mitochondrial Assay. Hearts were excised from three of four male Sprague–Dawley rats that were allowed free access to food and water prior to sacrifice by cervical dislocation. The mitochondria were then isolated by differential centrifugation as described.[14] The resulting mitochondrial pellet was then resuspended in 200 μL of buffer (containing 200 mM D-mannitol and 50 mM Trizma HCl at pH 7.4, 1.5 μL of a 4 mg/mL solution of rotenone, 1 μL of a 10 mg/mL solution of oligomycin, and 0.5 μL of a 12 mg/mL solution of antimycin A), and the suspension was warmed on a water bath to 28 °C. A 50-μL portion of a 2.8 mg/mL solution of *l*-carnitine and 250 μL of a solution of [^{14}C]-*l*-carnitine (corresponding to 5 μCi) were added, and the suspension was swirled to mix for 3 min. The mitochondria were then washed as described and resuspended as evenly as possible in 250 μL of buffer per heart used. Control tubes were prepared by combining 125 μL of buffer and 100 μL of water. Experimental tubes contained 125 μL of buffer and 100 μL of either a 7.5 or 125 mM solution of the compound being evaluated. Efflux of radioactivity was initiated by adding 25 μL of the mitochondrial suspension to each tube, and the samples were gently stirred or shaken in ice for 1 min. A 10-μL portion of mersalyl solution (26 mM mersalyl, 200 mM D-mannitol, 50 mM Trizma base) was then added to quench the reaction. The tubes were spun in an Eppendorf microcentrifuge for 90 s, and 200 μL of the supernatant from each sample was added to 10 mL of Aquasol II for scintillation counting. Total radioactivity was determined by counting 25 μL of the mitochondrial suspension directly. All DPM values (except total radioactivity) were multiplied by 1.3, since only 200 of 260 μL were counted. A value for percent efflux was determined by applying the following formula:

$$\% \text{ efflux} = 100 \times \frac{\text{DPM(exptl tube)} - \text{DPM(control tube)}}{\text{DPM(total radioact sample)} - \text{DPM(control)}}$$

Scintillation counting efficiency was 95%.

Registry No. **1**, 147-85-3; **2**, 4136-37-2; **3**, 51-35-4; **4**, 101198-81-6; **5**, 535-75-1; **6**, 498-95-3; **7**, 101312-27-0; (±)-**8**, 101198-32-7; (±)-**9**, 924-49-2; (±)-**10**, 101198-83-8; **11**, 101198-84-9; **12**, 636-82-8; **13**, 101198-85-0; **14**, 101198-86-1; palmitoyl chloride, 112-67-4; carnitine, 541-15-1; carnitine-acylcarnitine translocase, 56093-16-4.

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Allergenic α-Methylene-γ-butyrolactones. Study of the Capacity of β-Acetoxy- and β-Hydroxy-α-methylene-γ-butyrolactones To Induce Allergic Contact Dermatitis in Guinea Pigs

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(±)-Tulipalin B was prepared in six steps from phenyl sulfide and ethyl 2-bromopropionate. The sensitizing power in the skin of (±)-tulipalin A (**1a**) and B (**1b**) and of the β-acetoxy derivatives (**1c**) was studied. All are able to induce allergic contact dermatitis (ACD) and give cross-reactions. γ,γ-Disubstituted analogues (with a $-(CH_2)_5-$ chain in the γ-position) were synthesized and used to induce ACD in guinea pigs: they all were sensitizers and cross-reacted. However no cross-reaction was demonstrated between γ,γ-unsubstituted and γ,γ-substituted compounds showing a great specificity of ACD.

The α-methylene-γ-butyrolactone structural unit **1a** characterizes a number of natural compounds[1] with a variety of biological properties including the induction of *allergic contact dermatitis* (ACD). Among these sub-

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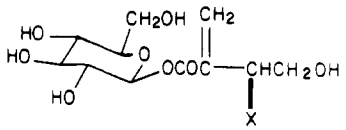
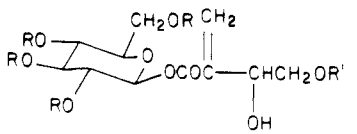
stances, the β-hydroxy derivative **1b**[2] has comparable properties although it has been claimed to be nonsensitizing.[3] Thus, tulip bulbs contain tuliposide A (**2a**),[4] precursor of tulipalin A (**1a**) (and the contact sensitizer responsible for "tulip fingers",[5] a dermatitis of the finger tips), and tuliposide B (**2b**), precursor of tulipalin B (**1b**), and supposedly unable to induce ACD.[3] The presence of a β-hydroxy group seems therefore to "quench" the ACD. Our interest in the sensitizing power of α-methylene-γ-butyrolactones[6] led us to devise syntheses of β-hydroxy- (and β-acetoxy-) α-methylene-γ-butyrolactones,[7,8] leading inter alia, to the first preparation of a derivative of tuliposide B[7] (**3**) and to the synthesis[9] of litsenolides C_1 and C_2 (**4** and **5**) isolated[1c] from *Litsea japonica*.

1a: X = H
b: X = OH
c: X = OAc

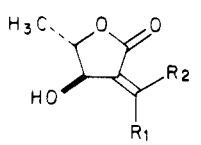
6a: X = H
b: X = OH
c: X = OAc



2a: X = H
b: X = OH



3: R = Ac, R' = CH_2Ph



4: R_1 = H, R_2 = $C_{13}H_{27}$
5: R_1 = $C_{13}H_{27}$, R_2 = H

We now describe the application of this synthetic scheme to a new synthesis of (±)-tulipalin B (**1b**) and to the study in the guinea pig of the compared sensitizing capacity of α-methylene-γ-butyrolactone (**1a** tulipalin A) and its γ,γ-disubstituted derivative **6a** and their β-hydroxy (**1b** tulipalin B; **6b**) and β-acetoxy (**1c**; **6c**) counterparts (see below).

Chemistry

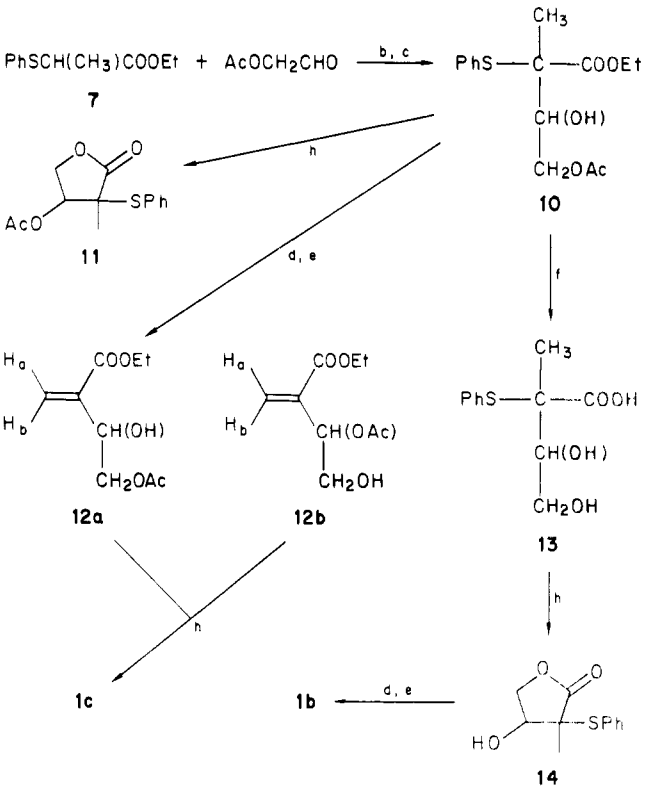
The synthesis of (±)-tulipalin B (**1b**) was first described in 1974:[10] it was obtained from tulipalin A (**1a**; α-methylene-γ-butyrolactone) by SeO_2 oxidation in a 5% yield. (-)-Tulipalin B was synthesized from (+)-glyceraldehyde[11] in 1980. We have described recently a new method for the preparation of β-acetoxy- and β-hydroxy-α-methylene-γ-butyrolactones based on the allylic sulfoxide–sulfonate rearrangement: (±)-tulipalin B and its β-acetyl derivative were prepared in this way.[7]

We have used the method we described recently[8] for the synthesis of the spiro β-acetoxy- and β-hydroxy-α-methylene-γ-butyrolactones **6b** and **6c**.

The condensation product was obtained directly in the cyclic form. Preparation of the parent compound **1b** (tulipalin B) was achieved through the same sequence. However, the phenylthiolactone **11** was not obtained directly, but the open derivative **10** was isolated instead (Scheme I). The starting α-acetoxyacetaldehyde was obtained[12] by the $Pb(OAc)_4$ oxidation of the enol ether of acetaldehyde, followed by acidic treatment to recover the unprotected aldehyde. The open-chain derivative **10** could be cyclized under acidic conditions into lactone **11**. The remainder of the synthesis was as shown in Scheme I.

The saponification of **12a,b** or lactone **1c** gave water-soluble substances that were difficult to extract. We preferred to saponify ($Ba(OH)_2$) the condensation product **10** first. Derivative **13** could be extracted and cyclized (*p*-TsOH treatment). Lactone **14** was formed with an overall 60% yield from compound **10**. Finally, (±)-tulipalin B could be obtained with a 90% yield from **14**.

Scheme I[a]



[a] Key: (a) THF, room temperature; (b) LDA, THF; (c) H_3O^+; (d) *m*-CPBA, CH_2Cl_2; (e) toluene, Δ; (d) $Ba(OH)_2$, H_2O, THF; (g) H_3O^+ (pH 4); (h) *p*-TsOH, $CHCl_3$.

Results and Discussion

Results are collected in Table I. The most striking finding is that tulipalin B (**1b**) *is a skin sensitizer*. The β-hydroxy lactone **6b** is also capable of eliciting a skin

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

	test compd[a]													
	1a (tulipalin A)		**1b** (tulipalin B)		**1c** (β-acetyl **1b**)		**6a**		**6b**		**6c**		controls[d]	
sensitizn[b] compd	av[c] skin reactn	no.[e]	av skin reactn	no.	av skin reactn	no.	av skin reactn	no.	av skin reactn	no.	av skin reactn	no.	av skin reactn	no.
1a	2.0	6/6	0.9	6/6	0.4	4/6	0	0/6	0	0/6	0	0/6	0	0/6
1b (β-OH)	1.1	6/6	1.0	6/6	0.3	4/6	0	0/6	0	0/6	0	0/6	0	0/6
1c (β-OAc)	1.1	6/6	1.0	6/6	0.8	6/6	0	0/6	0	0/6	0	0/6	0	0/6
6a	0	0/6	0	0/6	0	0/6	1.2	6/6	0.4	6/6	1.7	6/6	0	0/6
6b (β-OH)	0	0/6	0	0/6	0	0/6	1.7	6/6	0.7	6/6	1.8	6/6	0	0/6
6c (β-OAc)	0	0/6	0	0/6	0	0/6	0.8	6/6	0.5	6/6	3.0	6/6	0	0/6

[a] Tests were performed as follows: 25 μL of a 2% solution of the lactone in CH_2Cl_2 were deposited on a 2-cm^2 circular area (determined by a rubber stamp) on the shaved flank of the animals. All other lactones were tested at the same molar concentration. Tests were read after 24 h: 0 = no response; 0.5 = slight erythema covering part of the test area; 1.0 = erythema covering the whole area; 2.0 = erythema and swelling limited on the test area; 3.0 = erythema + swelling going well beyond the test area. [b] Sensitization was effected according to the Freund complete adjuvant test:[13] a stable emulsion of the hapten (5%) in a 1:1 FCA/saline mixture was injected intradermally in the shaved nuchal area of the animal; this injection was repeated two times on alternate days. After a 2-week rest, the animal was tested (see footnote *a*). [c] Average skin reaction obtained by adding all the numerical results of the tests (0–3) and dividing by the number of animals. [d] Controls received intradermal injection of a 1:1 FCA/saline emulsion on alternate days (three injections). [e] Number of sensitized animals.

response. It therefore seems that β-hydroxy-α-methylene-γ-butyrolactones are contact sensitizers, contrary to previous reports from the literature.[3] The agent responsible for tulip fingers (a contact dermatitis affecting finger tips in tulip bulb sorters) is thought to be tulipalin A (**1a**) in the form of a glucoside, tuliposide A (**2a**). Bulbs contain tuliposide B but in a much lower concentration (~10 times less) than tuliposide A (0.08% of the dry extract of the former compared to 0.82 for the latter). The amount of tuliposide B might not be sufficient to induce ACD to it. However, the absence of cross-sensitization between tulipalin A and tulipalin B[3] is difficult to explain. We have however found that guinea pigs sensitized to tulipalin A (**1a**) cross-reacted to tulipalin B (**1b**) and conversely.

A second remarkable result is the absence of cross-sensitivity reaction in the two series of compounds (the γ-unsubstituted lactones **1a-c** and the γ,γ-disubstituted lactones **6a–c**). This finding dramatically illustrates the importance of the global shape of the sensitizer and the specificity of the observed response. If the functional group (α-methylene γ-lactone) is necessary to induce ACD, probably through covalent bond formation with a protein carrier[14] it is not sufficient to warrant the existence of cross-sensitivity.

Long thought of as "functional group allergy" or "group sensitization" allergy, etc., cross-sensitivity appears more and more *specific*. Stereospecificity of ACD was first described by Mitchell with *d*- and *l*-usnic acids:[15] only the former was found to be sensitizing in forest workers. However, recent work on "lichen pickers dermatitis"[16] has shown that both *d*- and *l*-usnic acid were able to elicit a skin positive test. This probably reflects a case of simultaneous or "cosensitization" to two enantiomers. We have demonstrated recently that an almost total stereospecificity was observed in (+)- and (−)-frullanolide-sensitized guinea pigs: no cross-reaction existed in the two groups.[17] However, with smaller molecules, (+)- and (−)-α-methylene-γ-valerolactones, no specificity of the cutaneous response was observed.[18] Here perhaps the "functional group allergy" alone can explain the results: the haptens are such small molecules that the antigen is not sufficiently differentiated by the (+) vs. the (−) conjugation. Recent results from our laboratory, however, seem to show that enantiospecificity is related to the position (β-substituents are more specific) and the nature of the substituent (Papageorgiou and Benezra, unpublished results, 1985).

Finally, the results described here show (1) the importance of the global shape of the hapten in inducing ACD and (2) β-hydroxy-α-methylene-γ-butyrolactones are as capable as their β-H counterparts of inducing ACD.

Experimental Section

IR spectra were recorded on a Beckman Acculab spectrophotometer using $CHCl_3$ solutions; wavenumbers (reciprocal centimeters) are given. NMR spectra were recorded on a Perkin-Elmer R24B (60 MHz). Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0.0) as an internal standard; coupling constants (*J*) are expressed in hertz. Mass spectra were determined (ionization energy 70 eV) on a Thomson SCF THF 208 apparatus. Column chromatography was run on Merck silica gel, 60, 70–230 mesh, AST 11. The abbreviations used are as follows: THF, tetrahydrofuran; s, singlet; m, multiplet; d, doublet; t, triplet; q, quadruplet; *m*-CPBA, *m*-chloroperbenzoic acid; LDA, lithium diisopropyl amide.

Ethyl 4-Acetoxy-3-hydroxy-2-methyl-2-(phenylthio)-butyrate (10). To a solution of LDA (prepared from diisopropylamine (2.33 g, 0.023 mol) and 1.1 equiv of BuLi) was added dropwise, at −77 °C and under an argon atmosphere, a solution of ethyl 2-(phenylthio)propionate (4.77 g, 0.023 mol) in THF (10 mL). The mixture was stirred for 30 min, and a solution of α-acetoxyacetaldehyde (2.46 g; 0.024 mol) in THF (10 mL) was added dropwise. The reaction mixture was stirred for an additional 1 h at −77 °C and quenched with 10% aqueous HCl (to reach pH <7). Removal of THF followed by the usual workup with ether afforded a crude oil. After column chromatography on silica gel (eluent ethyl ether/hexane (1:1), the pure compound (3.95 g, 0.013 mol; 55% yield) was obtained as a liquid: IR ($CHCl_3$) 1730, 1740; ^1H NMR ($CDCl_3$) δ 1.16 (t, 3 H, $-CH_2CH_3$, J = 7.0), 1.38 (s, 3 H, C(CH_3)SPh), 1.96 (s, 3 H, CH_3CO), 3.80–4.30 (m, 4 H, $-CH_2CH_3$, CH_2OAc), 4.40–4.80 (m, 1 H, HOC*H*), 7.20–7.60 (m, 5 H, C_6H_5); MS, *m/e* 312 ($M^{+\cdot}$). Anal. ($C_{15}H_{20}O_5S$) C, H, S.

Ethyl 4-Acetoxy-3-hydroxy-2-methylenebutyrate (12a) and Ethyl 3-Acetoxy-4-hydroxy-2-methylenebutyrate (12b). To a solution of the sulfide **10** (3.47 g, 0.011 mol) in CH_2Cl_2 (20 mL) was added a solution of *m*-CPBA (2.07 g, 0.012 mol in CH_2Cl_2) at −10 °C. The mixture was stirred for 15 min and washed thoroughly with a saturated $NaHCO_3$ aqueous solution. The organic phase was dried over magnesium sulfate, the solvent was

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removed, and the crude sulfoxide (3.31 g, 0.010 mol; 90% yield) was refluxed in chloroform (100 mL). The reaction was complete after 1.5 h (as shown by TLC). After removal of the solvent and column chromatography on silica gel (eluent ethyl ether/hexane (75:25), products **12a** (1.69 g, 8.00 mmol) and **12b** (0.100 g, 0.500 mmol) were obtained as liquids: total yield 80% (based on the starting sulfide **10**); IR ($CHCl_3$) same functional groups absorptions at 3500–3600, 1740, 1710, 1630; ^1H NMR ($CDCl_3$) (**12b**) δ 1.30 (t, 3 H, CH_2, CH_3, J = 7.0), 2.10 (s, 3 H, OAc), 3.10–3.40 (m, 2 H, $-CH_2(OH)$), 5.82 (s, 1 H, H_b), 6.32 (s, 1 H, H_a). Spectral data for **12a** were identical with those of an authentic sample previously prepared in our laboratory by another method.[7] Compounds **12a** and **12b** were transformed by acid catalysis into lactone **1c**.[7]

β-Hydroxy-α-methyl-α-(phenylthio)-γ-butyrolactone (14). Sulfide **10** (0.85 g, 2.7 mmol) was dissolved in a mixture of THF (10 mL) and a saturated aqueous $Ba(OH)_2$ solution (30 mL). The reaction mixture was left for 4 h at room temperature; a solution of 2 N HCl was then added in order to adjust to pH 4. The THF was distilled off, and the aqueous phase wsa extracted with CH_2Cl_2. The organic layer was dried over magnesium sulfate, the solvent was removed, and the crude dihydroxyacid **13** was refluxed for a few minutes in $CHCl_3$ (50 mL) containing a catalytic amount of *p*-TsOH. Removal of the solvent and column chromatography on silica gel (eluent ethyl ether/hexane (75:25)) afforded lactone **14** (0.37 g, 1.6 mmol) in a 60% yield (from the starting sulfide **10**): mp 84–85 °C; IR ($CDCl_3$) 3400–3600, 1775; ^1H NMR ($CDCl_3$) δ 1.43 (s, 3 H, CH_3), 4.0–4.6 (m, 3 H, OCH_2, $-CH(OH)$), 7.3–7.8 (m, 5 H, SPh); MS, m/e 224 (M^+). Anal. ($C_{11}H_{12}O_3S$) C, H, S.

β-Hydroxy-α-methylene-γ-butyrolactone (1b, Tulipalin B). *m*-CPBA oxidation of lactone **14** (0.300 g, 1.32 mmol) and pyrrolysis of the crude sulfoxide was conducted under the same conditions as used for the sulfide **10**. Purification by silica gel column chromatography (eluent ethyl ether/hexane (75:25)) afforded lactone **1b** (0.135, 1.19 mmol; 90% yield from lactone **14**). The IR and NMR spectra were identical in all respects with data of the literature[10] (and with a sample prepared by another method[7]).

Biological Assays. Albino Himalayan spotted (Hoffmann La Roche, Füllingsdorf) female guinea pigs weighing from 300 to 500 g were sensitized by the FCAT method[13] 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 (a total of three injections were given). Six groups of six guinea pigs each were sensitized by an emulsion of a 1:1 FCA/saline mixture containing 0.22 mol/L of the haptens (compounds **1a–c** and **6a–c**).

After a 15-day rest, the elicitation reaction was achieved by an open epicutaneous test (OET): 25 μL of a 0.088 M (~2% w/v) solution of the hapten in a 1:1 mixture of ethanol and methylene chloride was deposited on the shaved flank of the animal (on a 2-cm^2 area delimited by a calibrated circular stamp). Skin reactions were read 24 h later against 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 (same procedure as above for elicitation) on FCA-injected controls. All compounds were nonirritating at a 2% concentration.

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Etodolac (1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic Acid): A Potent Antiinflammatory Drug. Conformation and Absolute Configuration of Its Active Enantiomer

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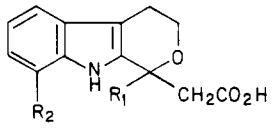
The active (+) enantiomer of the antiinflammatory agent etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic acid) has been assigned an *S* absolute configuration on the basis of a crystallographic analysis of the (*S*)-(−)-borneol ester of (−)-etodolac, and the conformation of etodolac has been determined by a crystallographic analysis of (±)-etodolac. Analyses of the solid-state conformation, as well as energy-minimized conformations obtained by molecular mechanics calculations, have failed to provide a basis for identifying a probable receptor-site conformation.

Etodolac (Ultradol) (**1**, 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid), has been shown to be a potent antiinflammatory drug with analgesic and antipyretic activity in animal models.[1,2] These properties have



1 (etodolac), $R_1 = R_2 = C_2H_5$
2 (prodolic acid), $R_1 = n\text{-}C_3H_7$, $R_2 = H$

also been demonstrated in man. Thus, etodolac is as efficacious as aspirin in rheumatoid arthritis patients at doses of 100 and 200 mg given twice daily.[3] In doses of 100 mg or higher, etodolac has demonstrated significant analgesia in patients following gynecologic, urologic, orthopedic, and oral surgery.[4–6] Etodolac has also been shown to reverse the skeletal changes associated with Freund's adjuvant arthritis in rats[7,8] and to retard the

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