from aqueous acetic acid gave **22**: mp 189–191 °C; NMR (Me_2SO) δ 8.13 (1 H, s), 7.88 (1 H, s), 7.58–6.38 (5 H, m). Anal. ($C_{10}H_8N_2O_2$) C, H, N.

5-[4-(Methylsulfinyl)phenoxy]-2(1*H*)-pyrimidinone (18). To a slurry of 1.17 g (0.005 mol) of **17** in 20 mL of methanol at 0 °C was added 1.175 g (0.0055 mol) of $NaIO_4$ in 10 mL of H_2O, and the slurry was stirred at 0 °C for 8 h and then for 20 h at 25 °C. The resultant slurry was diluted with H_2O and the methanol was removed in vacuo. Filtration gave 0.917 g (73%) of a white solid, mp 259–260 °C. Anal. ($C_{11}H_{10}N_2O_3S$) C, H, N.

Pharmacological Procedures. Guinea Pig Histamine Aerosol. Bronchodilator activity was evaluated according to the method of Van Arman, Miller, and O'Malley[13] in conscious female Reed–Willet guinea pigs (200–250 g) fasted overnight. One hour following po administration of vehicle, or the test drug dissolved in 1 N HCl or suspended in vehicle, each animal was challenged with histamine aerosol as follows: a 0.4% aqueous solution of histamine was placed in a Vaponephrine standard nebulizer (Vaponephrine Co., Edison, N.J.) and sprayed under an air pressure of 6 psi into a closed 8 × 8 × 12 in. transparent plastic container for 1 min. Immeditely thereafter, the guinea pig was placed in the container. The respiratory status (a reflection of bronchoconstriction) of the guinea pig after 1 min in the container was scored as follows: 0, normal breathing; 1, slightly deepened breathing; 2, labored breathing; 3, severely labored breathing and ataxia; 4, unconsciousness.

The percent inhibition values were derived by comparing the total score of a group of eight treated animals with that of a similar control group to generate a single value with no standard deviation. In order to assess the significance of a result, data on 11 groups of eight animals were recalculated by assigning a percent inhibition value to each animal (based on the score 0 = 100% inhibition, 1 = 75%, 2 = 50%, 3 = 25%, and 4 = 0) and where more than 90% of control scores were 4. Based on this calculation, the standard deviation of the mean value for eight animals was 5.33. As a result, it is likely that any two values differing by more than 11% would be significantly different based on the premise that values differing by more than 2 standard deviations represent different populations.

Cold-Restraint, Stress-Induced Gastric Ulceration. Ulcer protective effects were determined in rats using a modification of the method of Perkins.[15] Cold-restraint, stress-induced gastric ulceration was produced by immobilizing nonfasted, female, 90–120 g Sprague–Dawley rats in a supine position and placing them in a refrigerator at 12 °C for 3 h. Experimental drugs suspended in saline containing 10 g/L carboxymethylcellulose and 1 g/L Tween 80 were administered 3 h before the initiation of the stress period. At the conclusion of the ulcerogenic stress, the rats were sacrificed by cervical dislocation, their stomachs were removed, and the degree of gastric ulceration was determined. Differences in number of ulcers per animal were compared with Wilcoxon rank sum tests in order to calculate a p value. Compounds were considered active (+) if the decrease in number of ulcers per animal compared to control were significant at the $p \leq 0.05$ level.

(15) W. E. Perkins and L. Vars, *Br. J. Pharmacol.*, **47**, 847 (1973).

Synthesis of α-Methylene-γ-butyrolactones: A Structure–Activity Relationship Study of Their Allergenic Power

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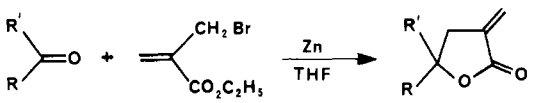
Thirty-five α-methylene-γ-butyrolactones have been prepared and their allergenic properties tested on the skin of guinea pigs experimentally sensitized to (a) alantolactone (**1**), (b) isoalantolactone (**2**), and (c) α-methylene-γ-butyrolactone (**3**). The two first groups of animals cross-react to lactones containing 9 to 18 carbon atoms but not to smaller α-methylene-γ-butyrolactones. Conversely, animals sensitized to α-methylene-γ-butyrolactone react only with α-methylene-γ-butyrolactones containing 6 and 7 carbon atoms. These results are discussed in relation with the allergic contact dermatitis mechanism.

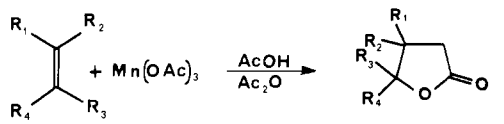
The biological properties of compounds containing the α-methylene-γ-butyrolactone moiety have been the focus of a considerable amount of work. Research has been focussed mainly on their cytotoxic, antitumoral,[1,2] antibacterial,[3] and plant growth inhibition activities.[4] These compounds, which occur naturally in many plants[5] (such as compositae, frullanaceae, magnoliaceae, etc.), can also cause severe allergic contact dermatitis (ACD) in man.[6]

We have been interested in this last property for some time. The mechanism of ACD has not been completely elucidated.[7] It is generally believed that an allergen (hapten), in order to become a true antigen, must bind to a protein (or another carrier). Why are some compounds allergenic while other are not? To approach the answer to this fundamental question, we have prepared a series of γ-monosubstituted and β,γ- and γ,γ-disubstituted α-methylene-γ-butyrolactones and tested their allergenic

Scheme I. Reformatsky Synthesis of γ-Substituted α-Methylene-γ-butyrolactone



Scheme II. Synthesis of Butyrolactones from Manganese Triacetate and Olefins



(1) C. H. Smith, J. Larner, A. M. Thomas, and S. M. Kupchan, *Biochim. Biophys. Acta*, **276**, 94 (1972).
(2) J. L. Hartwell and B. J. Abbott, *Adv. Pharmacol. Chemother.*, **7**, 117 (1969).
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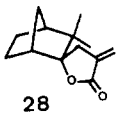
activity on experimentally sensitized guinea pigs. The *selectivity* of sensitization has been tested by using three groups of animals: one sensitized to alantolactone (**1**); another to isoalantolactone (**2**), two naturally occurring sesquiterpene lactones; and a third one to α-methylene-γ-butyrolactone (**3**) itself. This paper reports the synthesis and the immunological activity of a series of lactones.

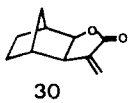
Results

Chemistry. Among the many available synthetic pathways leading to these compounds, the one-step Reformatsky reaction[8] from aldehydes or ketones and ethyl bromomethacrylate seemed attractive (Scheme I). These lead to γ-monosubstituted or γ,γ-disubstituted lactones. Most of the compounds reported in this paper have been prepared in this way.

Another pathway is the one-step formation of γ-butyrolactones from olefins and manganese(III) triacetate[9] (Scheme II). As attractive as it seems, this method is apparently not general and, in our hands, failed when there was an allylic hydrogen available. When oxidation can take place by abstraction of a hydrogen atom in an allylic position, the formed radical can be oxidized, yielding allylic acetates.[10a] Introduction of the methylene group[10b] leads to the desired lactones. Compounds **28** and **30** were prepared in this way and described elsewhere.[11]



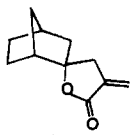
28



30

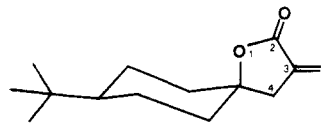
Structure of the Lactones. Combustion analyses, spectral properties (IR and NMR), and mass spectrometry (low and high resolution) confirm the assigned structures. There are several cases where isomers can be formed. These include the Reformatsky reactions of ethyl bromomethacrylate with norbornanone (**36**), 4-*tert*-butylcyclohexanone (**37**), 3-cholestanone (**38**), 1,4-cyclohexanedione (**39**), 3,7-bicyclo[3.3.0]octanedione (**40**), and α-decalone (**41**).

Norbornanone gives only one isomer, as shown by NMR using the shift reagent Eu(fod)$_3$. Because the products of most electrophilic[12] and radical[13] reactions in the bicyclo[2.2.1]heptane system are the results of an exo attack, the probable structure is **27** where the oxygen is endo.



27

The Reformatsky reaction with 4-*tert*-butylcyclohexanone (**37**) leads to an 85:15 mixture of isomers [as shown by the NMR spectrum in the presence of Eu(fod)$_3$]. A very recent publication by O'Donnell et al.[14] has described the same compounds. These authors were able to separate the two epimers and to assign the –O– axial isomer **23a** as the major one. The NMR spectrum shows the

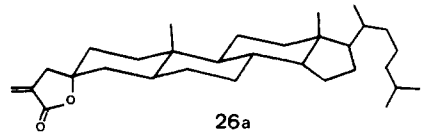


23a

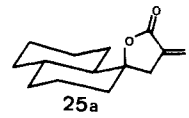
23b

major isomer to have the most shielded methylene group of the butyrolactone (C_4). Jones et al.[15] have demonstrated in related compounds that an axial methylene group absorbs at lower field than the equatorial one. The assignment for compound **23a** by O'Donnell et al. was confirmed by an X-ray determination. In the NMR (CCl_4) spectrum of the **23a/23b** mixture, the C_4 methylene protons of the major isomer absorbs at δ 2.62 and that of the minor isomer at δ 2.72. Addition of nucleophilic substances, such as Grignard reagents to ketone **37**, preferentially leads to –O– axial isomers,[16,17] in agreement with these results.

Similarly, 3-cholestanone leads to a 65:35 (NMR spectrum) mixture of isomers, the major one being **26a**. Al-



26a



25a

though Reformatsky reactions with steroidal ketones have been described recently, no configurational assignments were made.[18] Reaction with diones **39** and **40** leads to complex mixtures of lactones, while a 9:1 ratio of isomers is obtained from α-decalone **41**, the major one having the **25a** configuration. Compounds **34** and **35** were prepared from isoalantolactone as described under Experimental Section.

Biological Assays. Albino, white female Hartley and Himalayan spotted Füllingsdorf (from Hoffmann La Roche, Basel) guinea pigs weighing from 300 to 500 g were sensitized as described by Klečak:[19] 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 solutions were used: alantolactone (0.1%, w/v, in a 1:1 FCA–saline emulsion), isoalantolactone (0.2%, w/v, in a 1:1 FCA–saline emulsion), and α-methylene-γ-butyrolactone (5%, w/v, in a 1:1 FCA–saline emulsion).

After 15 days rest, the elicitation was conducted by an open epicutaneous test: 25 μL of a 1% solution of the lactones in CH_2Cl_2 was deposited on the clipped and shaved flank of the animal (on a 2-cm^2 surface by a standard circular stamp). 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

(8) E. Ohler, K. Reininger, and U. Schmidt, *Angew. Chem., Int. Ed. Engl.*, **82**, 457 (1970).
(9) (a) E. I. Heiba, R. M. Dessau, and W. J. Koehl, Jr., *J. Am. Chem. Soc.*, **90**, 5905 (1968); (b) E. I. Heiba and R. M. Dessau, *J. Am. Chem. Soc.*, **93**, 524 (1971); (c) E. I Heiba, R. M. Dessau, and P. G. Rodewald, *J. Am. Chem. Soc.*, **96**, 7977 (1974); (d) J. B. Bush, Jr., and H. Finkbeiner, *J. Am. Chem. Soc.*, **90**, 5903 (1968); (e) M. Okano, *Bull. Chem. Soc. Jpn.*, **49**, 1041 (1976).
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(18) K. H. Lee, E. S. Huang, C. Piantodosi, J. S. Pagano, and T. A. Geissman, *Can. Res.*, **31**, 1649 (1971).
(19) G. Klecak, H. Geleick, and J. R. Rey, *J. Soc. Cosmet. Chem.*, **28**, 53 (1977).

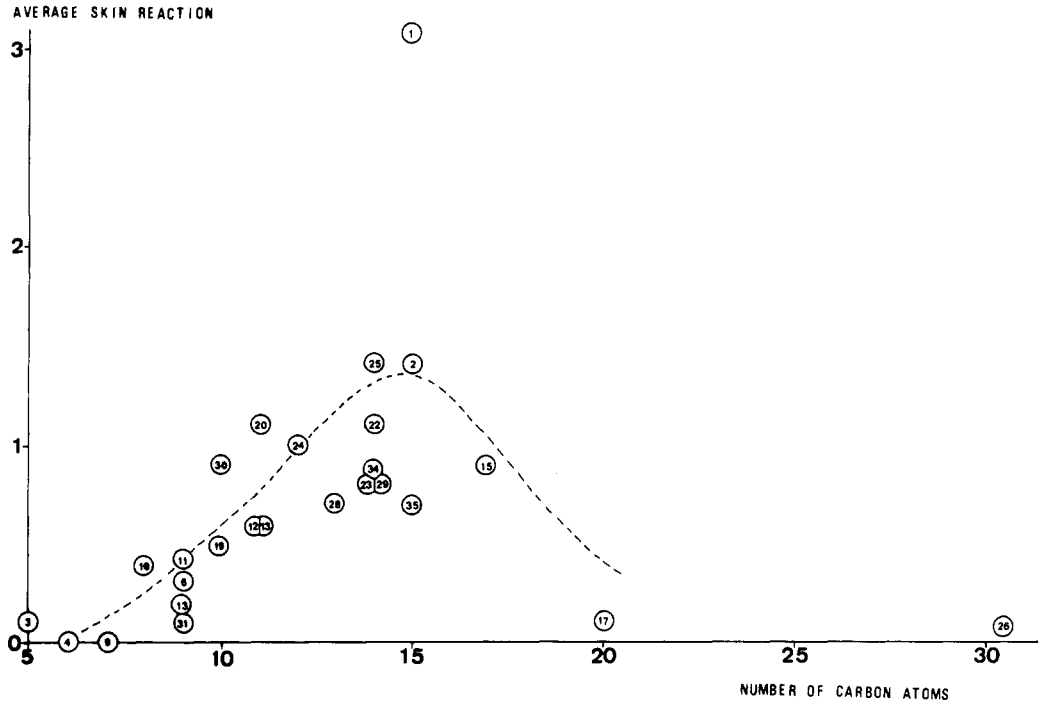


Figure 1. Plot of the average skin reaction of alantolactone-sensitized guinea pigs against the total number of carbon atoms.

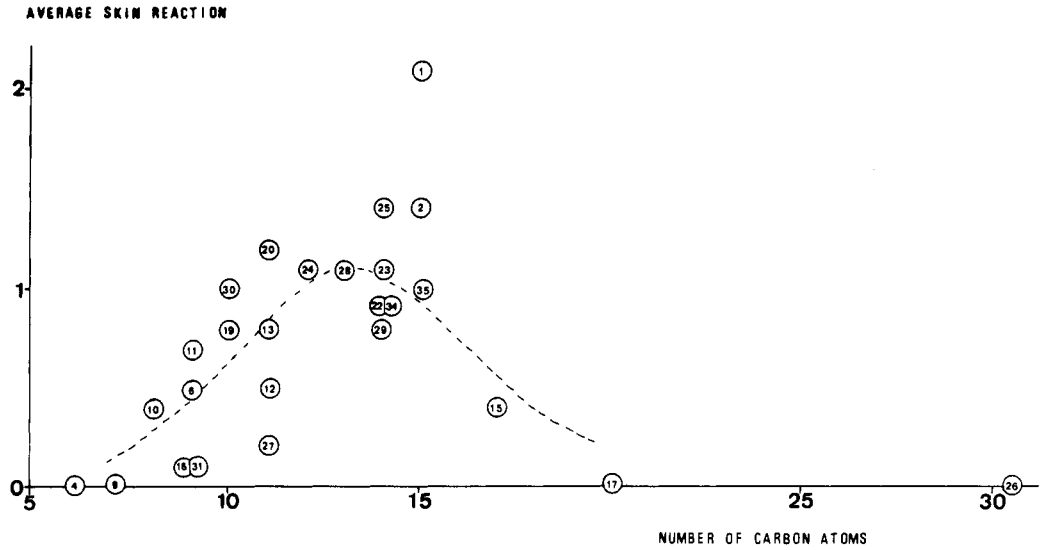


Figure 2. Plot of the average skin reaction of isoalantolactone-sensitized guinea pigs against the total number of carbon atoms.

the test area; 3 = erythema plus swelling going well beyond the test area; 4 = necrosis or ulceration.

Before any sensitization, irritation thresholds (primary toxicity) were determined on untreated animals (same procedure as above for the elicitation). Most of the compounds were nonirritating at the 1% concentration. Only compounds **7**, **8**, **14**, **16**, and **21** were toxic at this concentration and are not discussed in the structure–activity relationship of the lactones.

Results of the Elicitation Tests. The results of the elicitation tests are summarized in Table I. For each group of guinea pigs are given the number of guinea pigs with 3 to 0 reaction, the average test response, and the total number of allergic animals. An average reaction inferior to 0.5 can be considered as negative. That the sensitization has actually taken place is confirmed by the high ratio of positive tests to the primary sensitizer (i.e., the allergy-inducing agent).

In guinea pigs sensitized to the sesquiterpenes alantolactone (**1**) and isoalantolactone (**2**), cross-allergy is observed for compounds containing from 9 to 18 carbon atoms. The group sensitized to α-methylene-γ-butyrolactone only reacts to small molecules, i.e., γ-methyl- or γ,γ-dimethyl-α-methylene-γ-butyrolactones. Finally, bislactones **32** and **33** are totally inactive.

Discussion

A first striking result is the absence of cross-allergy between the sesquiterpene lactone-sensitized groups of guinea pigs and the one sensitized to a smaller molecule, α-methylene-γ-butyrolactone (**3**). There is no systematic reaction of this last group to α-methylene-γ-butyrolactone *derivatives*: this may seem surprising, since this moiety is the common denominator[6] of all natural lactones with sensitizing power. The rest of the molecule is therefore important. A structure–activity relationship on sesquiterpene lactones has been discussed recently.[20]

(20) E. Rodriguez, W. L. Epstein, and J. C. Mitchell, *Contact Dermatitis*, 4, 199 (1978).

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

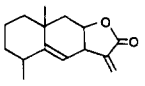
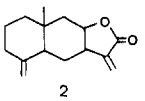
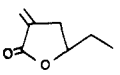
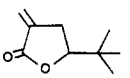
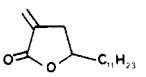
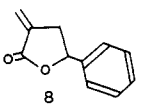
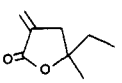
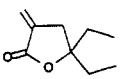
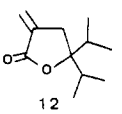
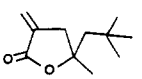
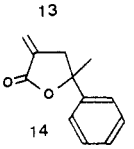
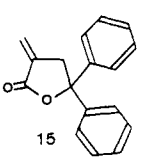
structures	no. of C atoms	guinea pigs sensitized to alantolactone		guinea pigs sensitized to isoalantolactone		guinea pigs sensitized to α-methylene-γ-butyrolactone	
		av[b] skin reaction	no. of sensitive animals	av[b] skin reaction	no. of sensitive animals	av[b] skin reaction	no. of sensitive animals
 1	15	3.0	7/7	2.1	8/8	0.0	0
 2	15	1.4	7/7	1.4	8/8	0.0	0
 3	5	0.1	1/7	nt[c]	nt[c]	1.9	10/10
 4	6	0.0	0	0.0	0	0.6	7/10
 5	7	nt[c]	nt[c]	nt[c]	nt	0.0	0
 6	9	0.3	3/7	0.5	5/8	0.1	1/10
 7	15	0.9[d]	7/7	1.2[d]	8/8	1.0[d]	10/10
 8	11	1.1[d]	7/7	1.5[d]	8/8	1.4[d]	10/10
 9	7	0.0	0/7	0.0	0/8	0.5	6/10
 10	8	0.4	5/7	0.4	6/8	0.0	0
 11	9	0.4	5/7	0.7	8/8	0.0	0
 12	11	0.6	6/7	0.5	6/8	0.0	0
 13	11	0.6	6/7	0.8	7/8	0.1	2/10
 14	12	1.1[d]	7/7	1.3[d]	8/8	0.4[d]	5/10
 15	17	0.9	7/7	0.4	5/8	0.0	0

Table I (*Continued*)

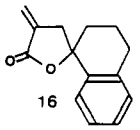
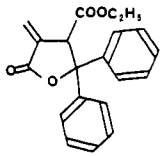
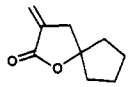
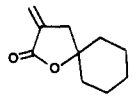
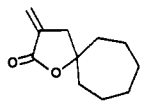
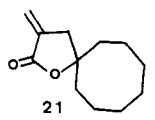
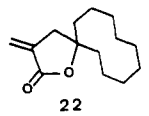
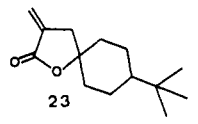
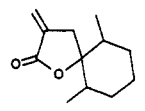
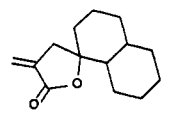
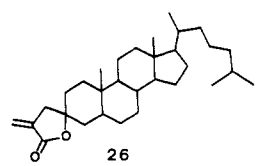
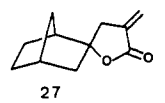
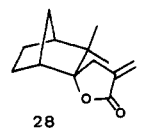
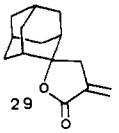
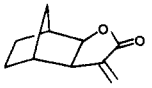
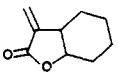
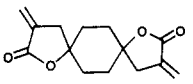
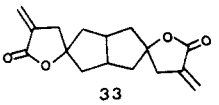
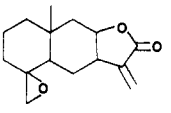
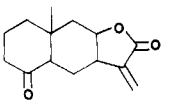
structures	no. of C atoms	guinea pigs sensitized to alantolactone		guinea pigs sensitized to isoalantolactone		guinea pigs sensitized to α-methylene-γ-butyrolactone	
		av[b] skin reaction	no. of sensitive animals	av[b] skin reaction	no. of sensitive animals	av[b] skin reaction	no. of sensitive animals
 16	14	1.7[d]	7/7	2.0[d]	8/8	0.9[d]	10/10
 17	20	0.1	1/7	0.0	0	0.0	0
 18	9	0.2	2/7	0.1	1/8	0.0	0
 19	10	0.5	5/7	0.8	8/8	0.1	2/10
 20	11	1.1	7/7	1.2	8/8	0.1	2/10
 21	12	0.9[d]	7/7	0.9[d]	8/8	0.0[d]	0
 22	14	1.1	7/7	0.9	7/8	0.0	0
 23	14	0.8	6/7	1.1	8/8	0.0	1/10
 24	12	1.0	7/7	1.7	8/8	0.0	0
 25	14	1.4	7/7	1.4	8/8	0.0	0
 26	31	0.1	1/7	0.0	0	0.0	0
 27	11	0.3	4/7	0.2	4/8	0.0	0
 28	13	0.7	7/7	1.1	8/8	0.0	0

Table I (*Continued*)

structures	no. of C atoms	guinea pigs sensitized to alantolactone: av[b] skin reaction	guinea pigs sensitized to alantolactone: no. of sensitive animals	guinea pigs sensitized to isoalantolactone: av[b] skin reaction	guinea pigs sensitized to isoalantolactone: no. of sensitive animals	guinea pigs sensitized to α-methylene-γ-butyrolactone: av[b] skin reaction	guinea pigs sensitized to α-methylene-γ-butyrolactone: no. of sensitive animals
 29	14	0.8	6/7	0.8	8/8	0.1	2/10
 30	10	0.9	7/7	1.0	8/8	0.1	2/10
 31	9	0.1	1/7	0.1	1/8	0.1	3/10
 32	14	0.0	0	0.1	1/8	0.0	0
 33	16	0.2	2/7	0.2	3/8	0.0	1/10
 34	15	0.7	7/7	1.0	8/8	nt[c]	nt[c]
 35	14	0.9	7/7	0.9	8/8	nt[c]	nt[c]

[a] Applied as 1% methylene chloride solution (0.025 mL on a 2-cm² area), except for compounds **1**, **2**, **34**, and **35** which were tested as 0.1% solutions. [b] This number refers to the average skin reaction ranging from 0 = no reaction, 1 = erythema on the test area, 2 = erythema plus swelling on test area, to 3 = erythema plus swelling going well beyond the test area. [c] nt = not tested. [d] Toxic.

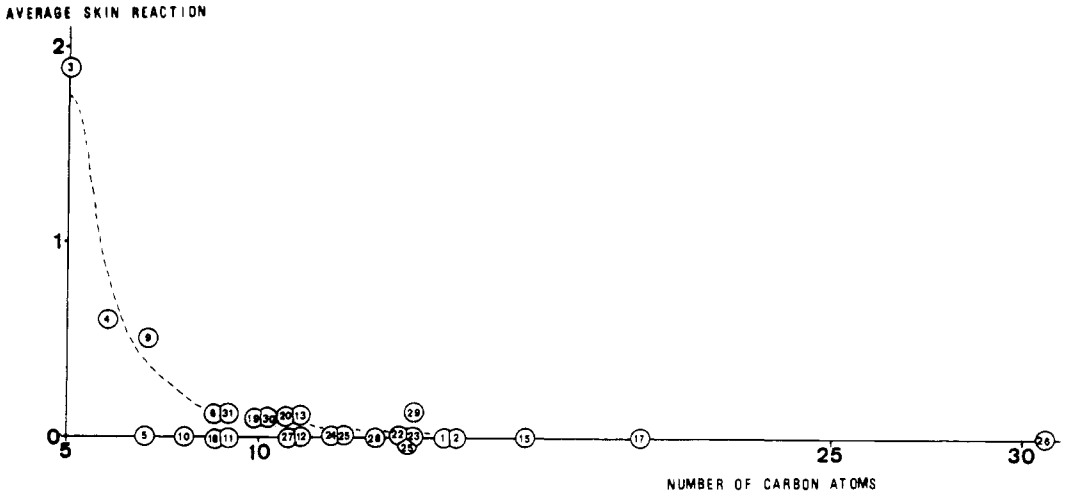


Figure 3. Plot of the average skin reaction of γ-methylene-γ-butyrolactone-sensitized guinea pigs against the total number of carbon atoms.

If one plots the average skin reaction against the total number of carbon atoms, one gets Figures 1–3. It is quite clear that cross-reactivity is best observed for compounds with a number of carbon atoms close to that of the primary sensitizer. This points out the *selectivity* of sensitization and confirms observations reported in the literature on

humans:[21] persons sensitive to sesquiterpene lactones are not allergic to tulip onion bulbs, for instance. For these, α-methylene-γ-butyrolactone (or its glucoside precursor) is claimed to be the sensitizer.[22] This selectivity of sensitization may be due to the presence of different "receptor sites" in the carrier or in the immunocompetent lymphocytes. For example, large sites (for C_{15} compounds) would not accomodate small molecules such as α-methylene-γ-butyrolactone and, conversely, small sites would not accomodate large molecules. Hydrophobic and hydrophilic interactions could also be of importance.

Another observation concerns alantolactone (1) and isoalantolactone (2). These are double-bond positional isomers and one could expect a good cross-reactivity between them. However, literature claims of the nonallergenic power of isoalantolactone[6,23] seemed to reveal a striking difference between these isomers. Our study shows that both compounds do indeed give cross-reactions, confirming earlier results from this laboratory. The situation of man and animal is parallel in this respect.[24] Nevertheless, let us note that isoalantolactone sensitization seems a little weaker than alantolactone senzitization. Also, if alantolactone-sensitized guinea pigs react reasonably well to isoalantolactone, isoalantolactone-sensitized animals react better to alantolactone than to the primary sensitizer!

These lactones might react differently because of discrepancies in penetration capacity, elimination rate, metabolism rate, solubility, etc. To check this last hypothesis, partition coefficients between isooctane and water for some compounds, and 1-octanol and water for others, were measured: no correlation seems to exist between the solubility and allergenic activity of these compounds. Finally, it is of interest to note that bislactones **32** and **33** are not allergenic at all.

Experimental Section

Infrared spectra were taken on a Beckman Acculab 1, using $CHCl_3$ or CCl_4 as solvent; wavenumbers are given in reciprocal centimeters. NMR spectra were registered on a Perkin-Elmer R 12 B (60 MHz) or R 32 (90 MHz) spectrophotometer. Gas chromatography was performed on a GIRDEL Model 300 chromatograph equipped with a flame-ionization detector. All compounds gave satisfactory IR and NMR absorption spectra and mass spectra; these will be reported elsewhere. The reported combustion analyses were within ±0.4% for all new compounds, except for compound **5** for which several analyses were unsatisfactory. However, all the other analytical methods, as well as IR, NMR, low- and especially high-resolution mass spectrometry, did agree with the proposed structure.

General Procedure for the Reformatsky Reaction. Ethyl bromomethacrylate was prepared in a 35 to 40% yield, according to Ferris.[25] Into a single-neck round-bottom flask dried in the oven and filled with argon were introduced, successively, 0.350 g (5.34 mmol) of freshly prepared zinc powder and 4.50 mmol of the aldehyde or lactone in freshly distilled tetrahydrofuran (15 mL). The mixture was stirred with a magnet and warmed at 60–70 °C. From the condenser, 0.965 g of ethyl bromomethacrylate (5.00 mmol) in THF (5 mL) was then added dropwise slowly, to avoid the reaction from becoming too fast. If the reaction did not start, a drop of a THF–iodine solution was added. All the γ-substituted lactones were prepared from 4.50 mmol of ketone or aldehyde, 5.00 mmol of ethyl bromomethacrylate, and 5.34 mmol of zinc, except for lactones **4**, **9**, **26**, **32**, **33**. These five compounds were prepared from 90.9, 27.5, 1.0, 2.25, and 2.25 mmol of ketone (or aldehyde), respectively, from 10.0, 4.50, 1.5, 5.00, and 5.50 mmol of ethyl bromomethacrylate, respectively, and from 10.7, 5.34, 1.5, 5.34, and 5.34 mmol of zinc, respectively.

After the addition was completed, the mixture was refluxed for 1 to 6 h, depending on the carbonyl compound, and cooled to room temperature. Filtration eliminated solid residues, and the filtrate was added to hydrochloric acid, 1 N (15 mL), and stirred for a few minutes. After extraction with methylene chloride (3 × 50 mL) and removal of the solvent, the crude product was deposited on a silica gel column (using 50 to 100 times the weight of adsorbent), prepared and eluted with an ether–petroleum ether (75:25) mixture. If necessary, thick-layer chromatography on silica gel prepared plates from Merck was used for further purification. A summary (yields and chemical formulas) is presented in Table II.

Table II. Preparation of α-Methylene-γ-butyrolactones: Formulas, Yields, and Physical Data

compd	formula[a,b]	anal.	yield, %	mp, °C, or oil
4	$C_6H_8O_2$[c,d]		65	oil
5	$C_7H_{10}O_2$[b]		23	oil
6	$C_9H_{14}O_2$	C, H	29	oil
7	$C_{16}H_{28}O_2$	C, H	84	oil
8	$C_{11}H_{10}O_2$[e]		96	oil
9	$C_7H_{10}O_2$[c,e]		64	oil
10	$C_8H_{12}O_2$	C, H	29	oil
11	$C_9H_{14}O_2$	C, H	23	oil
12	$C_{11}H_{18}O_2$	C, H	44	oil
13	$C_{11}H_{18}O_2$	C, H	27	oil
14	$C_{12}H_{12}O_2$[e]		75	oil
15	$C_{17}H_{14}O_2$[e,f]		71	104-105
16	$C_{14}H_{14}O_2$[e]		60	53-54
18	$C_9H_{12}O_2$[e]		89	oil
19	$C_{10}H_{14}O_2$[f]		37	oil
20	$C_{11}H_{16}O_2$[f]		42	oil
21	$C_{12}H_{18}O_2$	C, H	22	oil
22	$C_{14}H_{22}O_2$	C, H	31	oil
23	$C_{14}H_{22}O_2$[c]	C, H	68	61-65
24	$C_{12}H_{18}O_2$	C, H	71	oil
25	$C_{14}H_{22}O_2$	C, H	33	oil
26	$C_{31}H_{50}O_2$	C, H	27	oil
27	$C_{11}H_{14}O_2$	C, H	29	oil
29	$C_{14}H_{18}O_2$	C, H	24	161-162
32	$C_{16}H_{18}O_2$[g]		33	*h*
33	$C_{14}H_{16}O_4$[b,g]		33	*h*
34	$C_{15}H_{20}O_3$	C, H	71	126-127
35	$C_{14}H_{18}O_3$	C, H	91	153-155

[a] As determined by combustion analysis. [b] As determined by high resolution mass spectrometry. [c] Reference 14. [d] Reference 26. [e] Reference 8. [f] Reference 27. [g] Reference 28. [h] Crystalline mixture.

The chemical purity of the known compounds **4**, **9**, and **14–20** was checked by gas chromatography (GIRDEL 3000), using a 20-m capillary column packed with OV17 (internal diameter 0.25 mm). The oven temperature was 120 to 280 °C, depending on the lactone, and the injection temperature was 150 to 280 °C.

Preparation of 4,14-Epoxyisoalantolactone (34). To a solution of isoalantolactone (1.0 g, 4.3 mmol) in methylene chloride (4 mL) was added dropwise a solution of *m*-chloroperbenzoic acid (1.2 g, 4.8 mmol) in methylene chloride (10 mL). No rise of temperature was noted. The mixture was allowed to stand at room temperature for 24 h, and the precipitated *m*-chlorobenzoic acid was collected by filtration and washed with methylene chloride

(21) C. Benezra, "Actualités Allergologiques, Soc. Francaise d'Allergologie", Xe Journées Nationales, Strasbourg, France, 1972, expansion scientifique, p 57.
(22) (a) G. A. N. Verspyck Mijnssen, *Br. J. Dermatol.*, 81, 737 (1969); (b) B. H. Bergman, J. C. M. Beijersbergen, J. C. Overeem, and A. Kars-Sijpesteijn, *Recl. Trav. Chim. Pays-Bas*, 86, 709 (1967); (c) U. W. Bongersma-Oosterhoff, *ibid.*, 86, 705 (1967).
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(25) A. F. Ferris, *J. Org. Chem.*, 20, 780 (1955).
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(2 × 10 mL). Usual workup of the filtrate gave a solid, which, upon recrystallization in ether, gave compound **34** (762 mg): mp 126–127 °C; IR (CCl_4) 1765 (C=O lactone), 1670 (C=C) cm^{-1}; NMR δ ($CDCl_3$) 0.99 (3 H, s, C_{10} CH_3), 2.54 (AB q, 2 H, C_4 OCH_2, J_{AB} = 4.9 Hz), 2.87 (1 H, m, H_7), 4.43 (H, br t, H_8, $J_{7,8}$ = 5.3 Hz), 5.51 (1 H, s, H_{13a}), 6.07 (1 H, s, H_{13b}); MS *m/e* 248 (M^+), 238, 137, 107. Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.49; H, 8.05. Found: C, 72.41; H, 8.03.

Preparation of 4-Ketonorisoalantolactone (35). To a solution of **34** (1.03 g, 4.14 mmol) in anhydrous ether (3 mL) and chloroform (1 mL) at room temperature was added a solution of H_5IO_6 (0.343 g) in anhydrous ether (60 mL). The mixture was stirred for 48 h in the dark, extracted with ether, washed with sodium metabisulfite (10% solution), sodium bicarbonate (5% solution), and water, dried, and evaporated to dryness. The residue was chromatographed on a silica gel column (25 g, 24-cm high with a 1.8-cm diameter). Elution with ether and recrystallization from ethanol gave **35** (0.885 g) as colorless needles: mp 153–155 °C; IR ($CHCl_3$) 1760 (C=O lactone), 1720 (C=O ketone), 1670 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 0.89 (3 H, s, C_{10} CH_3), 2.1–2.4 (3 H, m, CH_2COCH), 2.93 (1 H, m, H_7), 5.60 (1 H, s, H_{13a}), 4.47 (1 H, dt, H_8, $J_{7,8}$ = 4.7 Hz, $J_{7,9}$ = 2 Hz); MS *m/e* 234 ($M^{+\cdot}$), 219, 191, 111. Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.69; H, 7.68. Found: C, 71.71; H, 7.78.

Acknowledgment. Financial support of this work from the Institut National de la Santé et de la Recherche Médicale (Contract libre 77-1-0973) and the Délégation Générale à la Recherche Scientifique et Technique is acknowledged. We also thank Drs. S. G. Klečak and L. Kinnen (Hoffman La Roche, Basel) for providing us with "Füllingsdorf" Himalayan spotted guinea pigs and J. P. Corbet for providing us with α-methylene-γ-butyrolactone. An "allocation d'études" (DGRST) to J. L. Stampf is acknowledged.

Metabolic N-Hydroxylation. Use of Substituent Variation to Modulate the in Vitro Bioactivation of 4-Acetamidostilbenes

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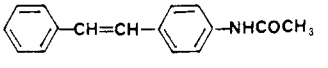
Received December 19, 1979

N-Hydroxylation is an obligate step in the bioactivation of carcinogenic aryl amides. Previous reports from this laboratory demonstrated that variation of the 4′ substituent of *trans*-4-acetamidostilbene (**1**) has a marked effect on the rate of its in vitro microsomal N-hydroxylation. In order to further investigate the effects of electronegative and aliphatic substituents, the 4′-CN, 4′-CH_3, 4′-$C(CH_3)_3$, and 4′-CF_3 analogues of **1** were synthesized and subjected to metabolic transformation by hamster hepatic microsomes. Each compound was synthesized in radiolabeled form, and the metabolites were identified and quantified by TLC, mass spectrometry, and liquid scintillation counting. The V_{max} for N-hydroxylation of the 4′-CN analogue was 24% and the K_m was 11% of that of **1**. The glycolamide was a minor metabolite of the 4′-CN compound. The principal metabolite of the 4′-CH_3 compound was the 4′-CH_2OH derivative, the N-hydroxylated product being formed in small quantities. Similarly, the 4′-$C(CH_3)_3$ analogue was metabolized to yield *trans*-4′-[2-(hydroxymethyl)-2-propyl]-4-acetamidostilbene (**26**) along with trace quantities of the hydroxamic acid. The 4′-CF_3 substrate yielded small amounts of the N-hydroxylated material as the only detectable metabolite. Thus, introduction of a 4′ substituent into **1** resulted in a decreased rate of N-hydroxylation for all compounds studied. The reduction in N-hydroxylation depends on both the physicochemical properties of the 4′ substituent and upon the susceptibility of the substituent to metabolic oxidation.

The *N*-aryl amide class of chemical carcinogens is one of the several types of organic chemical carcinogens which require metabolic activation before carcinogenic activity can be manifested. The initial process in the bioactivation of carcinogenic *N*-aryl amides (I) is metabolic hydroxylation of the amide nitrogen atom to yield an *N*-arylhydroxamic acid (II).[3] Although N-hydroxylation is an

obligate step in the induction of carcinogenesis by *N*-aryl amides, the available evidence indicates that, in most cases, further bioactivation of the resultant hydroxamic acid is required. Indeed, *N*-arylhydroxamic acids are potentially capable of undergoing several types of "second step" biotransformations to form electrophilic intermediates which are believed to covalently bind to critical nucleophilic target sites in cellular macromolecules and thereby initiate the carcinogenic process.[4,5]

Because N-hydroxylation, which is mediated by the microsomal mixed function oxidases, is the single biochemical activation process which is common to all members of the *N*-aryl amide class of chemical carcinogens, it is reasonable to expect that an understanding of the molecular characteristics which influence the tendencies of these compounds to serve as substrates for the microsomal *N*-hydroxylase systems would contribute to the development of means to reduce their potential for being metabolically converted to carcinogens. Such information should be useful in the design of new drugs, pesticides, herbicides, and other environmental chemicals to which man and animals are exposed. Previous work conducted in this laboratory demonstrated that variation of the 4′ substituent of *trans*-4-acetamidostilbene (**1**) has a marked



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effect on its rate of in vitro microsomal N-hydroxylation.[6,7]

(1) Department of Toxicology and Pathology, Mead Johnson Co., Evansville, IN 47721.
(2) College of Pharmacy, Yeungnam University, Gyongsan 632, Korea.
(3) J. A. Miller, *Cancer Res.*, **30**, 559 (1970).
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