Saturated Analogues of Poison Ivy Allergens. Synthesis of *trans,trans*- and *cis,trans*-3-Alkyl-1,2-cyclohexanediols and Sensitizing Properties in Allergic Contact Dermatitis

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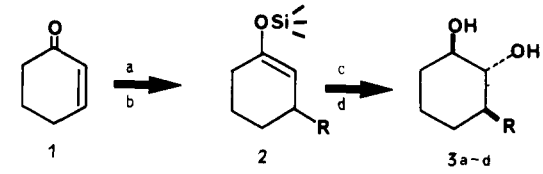
Saturated analogues of poison ivy and oak allergens (3-alkylcatechols), i.e. *trans,trans*-3-alkyl-1,2-cyclohexanediols (alkyl = CH_3, n-C_5H_{11}, n-$C_{10}H_{21}$, n-$C_{15}H_{31}$), have been prepared and used to sensitize guinea pigs. Only long-chain derivatives (carbon chain length > C_{10}) are contact sensitizers. The sensitized animals cross-react to PDC (i.e. pentadecylcatechol, one of the allergens of poison ivy), but the converse is not true (PDC-sensitized animals do not react to cyclohexanediols). *cis,trans*-3-*n*-Pentadecyl-1,2-cyclohexanediol has also been synthesized and shown to be a sensitizer. There is not cross-reaction between *trans,trans*- and *cis,trans*-3-*n*-pentadecylcyclohexanediols, excluding a common skin metabolite.

Allergic contact dermatitis to toxicodendron (poison ivy, oak, sumac) is a major cause of morbidity in the United States (80% of the U.S. population is allergic to them[1]). The allergenic principles contained in the resin of these plants, called urushiol, are a group of catechols[2] differing mainly in the length and degree of unsaturation of the 3-*n*-alk(en)yl side chain. In the last years, several attempts have been made to induce immune tolerance to these compounds in the guinea pig, in particular by the intravenous injection of urushiol derivatives,[3] 3-*n*-pentadecylcatechol coupled to autologous blood cells,[4] or incorporated into liposome.[5] However, since urushiols are highly toxic and potent primary irritants, their use for inducing tolerance in nonsensitive humans is heavily compromised. It would therefore be useful to make nontoxic analogues of these products, another approach to tolerance being the use of modified related substances, i.e. poor sensitizers. Thus sodium dinitrobenzenesulfonate[6] or dinitrothiocyanobenzene[7] induce tolerance to dinitrofluorobenzene.

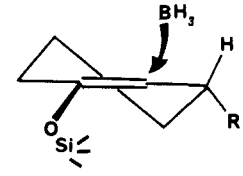
It is generally admitted that low-molecular-weight compounds are not immunogenic unless covalently attached to proteins.[8] Unfortunately, high chemical reactivity of allergens often accompanies their toxicity, as is the case, for instance, for alkylcatechols. An exception to the rule that immunogenic conjugates may only be formed by covalent binding appears to be the example of some low-molecular-weight compounds strongly bound to cell membranes. This is the case for some metal salts,[9] or picric acid, a low-molecular-weight sensitizer, which is an example of a compound that, although not covalently associating with carriers, gives rise to an immunogenic complex.[10] Experiments using methylated albumins or electrostatically charged polypeptides as carriers indicate, however, that molecules *strongly* attached to such carriers by *noncovalent* bonds may also behave as immunogens.[11]

Scheme I[a]



[a] (a) R_2CuLi, (b) $ClSiMe_3$, (c) BH_3-Me_2S, (d) H_2O_2, NaOH. For **3**: **3a**, R = CH_3; **3b**, R = n-C_5H_{11}; **3c**, R = n-$C_{10}H_{21}$; **3d**, R = n-$C_{15}H_{31}$.

Scheme II



Urushiol is an extremely lipophilic hapten and concentrates readily in cell membranes. It was shown to be highly soluble in but not covalently bound to the cell membrane, since, although it was not removed from the membrane by aqueous extraction, it could nevertheless be extracted with Me_2SO.[12] Urushiol-specific blastogenesis can be elicited from cultured peripheral blood lymphocytes by noncovalently bound hapten associated with the membranes because of its lipophilic character.[13] Finally Byers et al. have shown that insertion of lipophilic molecules into the lipid phase of tumor cells can induce a modification of tumor immunogenicity.[14]

It therefore seemed of interest to synthesize nonelectrophilic and nontoxic compounds capable of forming strong noncovalent bonds. This paper reports the preparation of such compounds, alkylcyclohexanediols, saturated analogues of poison ivy and poison oak pentadecylcatechols, as well as a study of their sensitizing power in guinea pigs.

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Table I. Characteristic NMR Data

R, trans,trans series	yield,[a] %	^{1}H NMR[b], ppm		coupling constants, Hz				^{13}C NMR[b]	
		H_1	H_2	J_{12}	J_{23}	J_{16}	$J_{16'}$	C_1	C_2
3a, CH_3	70	3.41	3.00	9.3	9.3	10.3	4.6	75.38	81.52
3b, n-C_5H_{11}	65	3.38	3.00	9.1	9.1	10.2	4.6	75.48	79.69
3c, n-$C_{10}H_{21}$	58	3.38	3.02	9.3	9.3	10.2	4.6	75.55	79.82
3d, n-$C_{15}H_{31}$	55	3.38	3.01	9.3	9.3	10.1	4.6	75.56	79.81
R, cis,trans series	yield,[a] %	^{1}H NMR,[b] ppm		coupling constants, Hz				^{13}C NMR[b]	
		H_1	H_2	$J_{1'2}$	J_{23}	$J_{1'6}$	$J_{1'6'}$	C_1	C_2
5, n-$C_{15}H_{31}$	65	3.94	3.28	3.0	9.1	2.8[c]	5.2[c]	69.80	75.68

[a] Isolated compound; yield calculated relative to the starting material. [b] In δ units relative to Me_4Si (δ = 0). [c] Difficult to measure.

Table II. Results of Open Epicutaneous Tests on Guinea Pigs

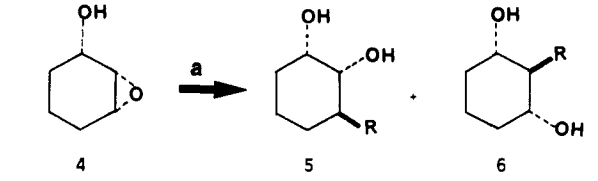
sensitizer[a]	compd tested (μmol)	no. of animals reading with a test intensity of				av skin reactn	no. of positive animals
		2	1	0.5	0		
3c, R = n-$C_{10}H_{21}$[b]	**3**, R = n-$C_{10}H_{21}$ (3.8)	0	1	3	4	0.3	4/8
3c, R = n-$C_{10}H_{21}$[b]	**3**, R = n-$C_{10}H_{21}$ (14)	2	2	2	2	0.9	6/8
3c, R = n-$C_{10}H_{21}$[c]	**3**, R = n-$C_{10}H_{21}$ (3.8)	0	2	4	2	0.5	6/8
3c, R = n-$C_{10}H_{21}$[c]	**3**, R = n-$C_{10}H_{21}$ (7.6)	0	5	1	2	0.7	6/8
3c, R = n-$C_{10}H_{21}$[c]	**3**, R = n-$C_{10}H_{21}$ (14)	2	2	3	1	0.9	7/8
3c, R = n-$C_{10}H_{21}$[c]	**3**, R = n-$C_{15}H_{31}$ (7.6)	0	1	6	1	0.5	7/8
3d, R = n-$C_{15}H_{31}$[b]	**3**, R = n-$C_{15}H_{31}$ (3.8)	0	4	8	4	0.5	12/16
3d, R = n-$C_{15}H_{31}$[b]	**3**, R = n-$C_{15}H_{31}$ (7.6)	0	12	2	2	0.8	14/16
3d, R = n-$C_{15}H_{31}$[b]	**7** (0.013)	0	1	6	1	0.5	7/8
3d, R = n-$C_{15}H_{31}$[c]	**3**, R = n-$C_{15}H_{31}$ (3.8)	1	9	4	2	0.8	14/16
3d, R = n-$C_{15}H_{31}$[c]	**3**, R = n-$C_{15}H_{31}$ (7.6)	2	11	2	1	1.0	15/16
3d, R = n-$C_{15}H_{31}$[c]	**3**, R = n-$C_{10}H_{21}$ (7.6)	0	5	1	2	0.6	6/8
3d, R = n-$C_{15}H_{31}$[c]	**5**, R = n-$C_{15}H_{31}$ (7.6)	0	0	1	7	0.1	1/8
5, R = n-$C_{15}H_{31}$[b]	**5**, R = n-$C_{15}H_{31}$ (3.8)	0	0	5	3	0.3	5/8
5, R = n-$C_{15}H_{31}$[b]	**5**, R = n-$C_{15}H_{31}$ (7.6)	0	1	6	1	0.5	7/8
5, R = n-$C_{15}H_{31}$[c]	**5**, R = n-$C_{15}H_{31}$ (3.8)	0	2	4	2	0.5	6/8
5, R = n-$C_{15}H_{31}$[c]	**5**, R = n-$C_{15}H_{31}$ (7.6)	0	5	2	1	0.8	7/8
5, R = n-$C_{15}H_{31}$[c]	**3**, R = n-$C_{15}H_{31}$ (7.6)	0	0	1	7	0.1	1/8
7 (PDC)[b]	**7** (0.013)	1	5	2	0	1.0	8/8
	3, R = n-$C_{15}H_{31}$ (7.6)	0	0	0	8	0	0/8

[a] No sensitization was obtained with 3-methyl- (**3a**, R = CH_3) and 3-pentyl-1,2-cyclohexanediols (**3b**, R = n-C_5H_{11}). In the two groups of eight guinea pigs used, no animal reacted. [b] Sensitized by the FCAT method, using three injections (each other day) of a 1:1 FCA/saline emulsion of the hapten. [c] Sensitized by the FCAT method, using five injections.

Chemistry. The model chosen, as close as possible to 3-alkylpyrocatechols, was 3-alkyl-1,2-cyclohexanediols. The spatial arrangement that seemed closest to the *o*-diphenol appeared to be *all-trans*-3-alkyl-1,2-cyclohexanediols. To our knowledge, the only synthesis of pure *all-trans*-3-alkyl- (in this instance, 3-methyl) cyclohexanediols was described by Klein and Dunkelblum,[15] their starting material being commercially available 3-methyl-2-cyclohexenone. The same authors have described another synthesis of this compound from cyclohexenone[16] unfortunately leading to a mixture of trans,trans and trans,cis isomers. We have now devised a synthesis based on the Michael addition of R_2CuLi (or RMgX with CuI catalysis) to 2-cyclohexenone (1). In the first process, to a solution of R_2CuLi (made in situ from RLi and CuI) in THF cooled at −60 °C was added 2-cyclohexenone. The resulting enolate was quenched with chlorotrimethylsilane and extracted. The crude [(trimethylsilyl)oxy]cyclohexene was then added to a solution of BH_3–Me_2S complex in THF.[17] In the second process, R_2CuLi was replaced by RMgBr (in the presence of CuI) (Scheme I).

Only one stereomer (the all-trans derivative) was obtained in every instance, as evidenced by GC and NMR spectroscopy. The configuration was secured by the magnitudes of J_{12} and J_{23}, characteristic in cyclohexane systems of a diaxial arrangement[18] (Table I). Because of the presence of the bulky trimethylsiloxy (OTMS) group, diborane attack occurs exclusively from the nonhindered face (Scheme II). All compounds, except for 3-methyl-1,2-cyclohexanediol (**3a**), are new.

Scheme III[a]



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[a] a = RMgBr (+ CuI).

Compared to the one-pot reaction of Michael addition/hydroboration of cyclohexenones, the yields are greatly improved.

We have also prepared *cis,trans*-3-pentadecyl-1,2-cyclohexanediol, which was needed (see discussion below) in order to eliminate one possible mechanism of sensitization to cyclohexanediols. The key material was *cis*-2,3-epoxycyclohexanol. The classical way for the synthesis of this compound was direct epoxidation (*m*-chloroperbenzoic acid) of 2-cyclohexenol.[19] However, a 9:1 mixture of cis/trans isomer was obtained. In order to obtain di-

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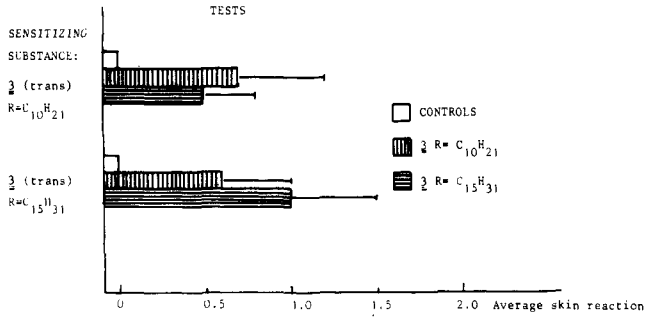


Figure 1. Compared skin sensitivity to *trans,trans-n*-decyl- and *n*-pentadecylcyclohexanediols in guinea pigs.

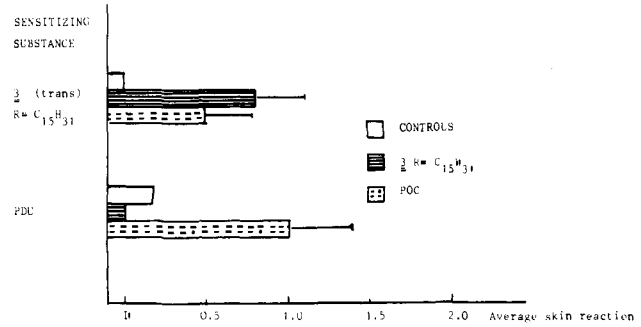


Figure 2. Compared skin sensitivity to *trans,trans-n*-pentadecylcyclohexanediol and pentadecylcatechol (PDC).

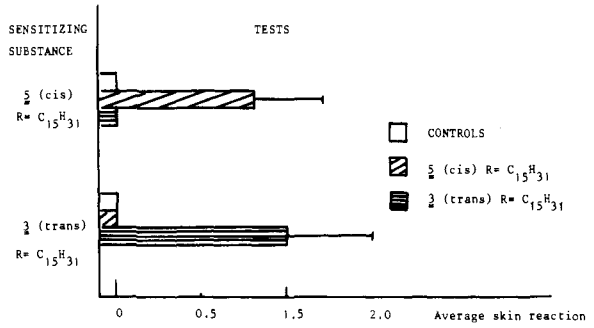


Figure 3. Compared skin sensitivity to *cis,trans-* and *trans,-trans-n*-pentadecylcyclohexanediols.

rectly pure isomers without time-consuming purification (isomers with long hydrocarbon chains are difficult to separate), we used the Bongini et al. procedure[20] as modified by Lipschutz,[21] namely, iodocarbonation of 2-cyclohexenol, followed by $MeOH/K_2CO_3$ treatment, leading to *cis*-2,3-epoxycyclohexanol (**4**) in 65% overall yield. Opening of *cis*-2,3-epoxycyclohexanol by RMgX (with CuI catalysis) led to *cis,trans*-3-alkyl-1,2-cyclohexanediol **5**, which was easily purified by making *cis*-diol acetonide (Scheme III).

Results and Discussion

The results of experimental sensitization of guinea pigs to *trans,trans*-1,2-cyclohexanediols appear in Table II. The first striking result is the importance of the side-chain length as far as the sensitizing power is concerned: when the number of carbons was inferior to 10, no sensitization was obtained. Thus, 3-methyl- and 3-pentyl-1,2-cyclohexanediols were nonsensitizers. This was already known for 3-alkylcatechols,[22] where an optimum 11–15 carbon chain was found.

Importance of the 3-Alkyl Side Chain Length. None of the four 3-alkyl derivatives (**3a–d**) showed any skin toxicity or primary irritation effects. As far as the pentadecyl compound is concerned, this is in contrast with the high toxicity found for 3-alkylcatechols by Baer et al.[23]

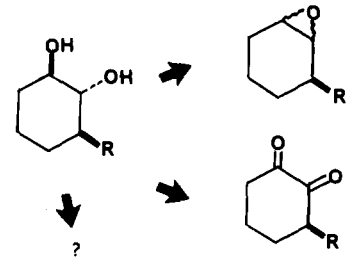
In the cyclohexanediol series, only C_{10} and C_{15} derivatives were able to induce ACD (allergic contact dermatitis). They, however, can be considered as weak sensitizers as compared to their catechols counterparts, a 300 times higher dose being required to elicit a positive skin test reaction. In order to make sure that 3-methyl- and 3-pentylcyclohexanediols were nonsensitizers, a much higher dose was used in an attempt to elicit a positive reaction without result.

Boosting the sensitization by two injections of alkylcyclohexanediol in FCA (Freund complete adjuvant, a suspension of killed mycobacteria in mineral oil) 1 week after the first test increased the sensitivity of the animals and the results were even more homogeneous (Table II).

Cross-sensitization reactions between C_{10} and C_{15} diols (Figure 1) show results analogous to those with Baer's corresponding catechols.[22]

Cross-Sensitization Reaction with Catechols. A striking result is the "one-way" cross-reaction between 3-PDC (or urushiol) sensitized animals and the 3-alkyl-

Scheme IV. Possible Metabolites of *trans,trans*-3-Alkylcyclohexanediols



cyclohexanediols. Thus, cyclohexanediol-sensitized animals react to PDC and to urushiol, but the converse is not true, i.e. PDC- and urushiol-sensitized guinea pigs do not react to cyclohexanediols (Figure 2). Such one-way cross-reactions are known in contact dermatitis.[24] Strong allergens only generate a few clones. Clones of cross-reacting cells are therefore strong when weak-sensitizer animals are elicited with a strong hapten, as observed.

"Lipophilic" or "Electrophilic" Mechanism? It is generally admitted that, in order to become an antigen, hapten binds to epidermal proteins with the formation of a covalent bond.[8] In the case of 3-alkylcatechols, general belief is that in vivo oxidation of these derivatives into electrophilic *o*-quinones precedes nucleophilic attack (though SH or NH_2 groups) by skin protein. The role of the side chain is, however, very important as shown by Dawson's results.[22] Catechols with long chains (C_{11} seems the optimum length) are stronger allergens. A possible explanation for this is that the chain is inserted into the cell membrane (possibly Langerhans cells) and the hydrophilic part emerges from it. In support for this hypothesis, double-headed haptens[25] have been prepared in this laboratory and shown to induce ACD in guinea pigs: only the pyrocatechol "end" was recognized.

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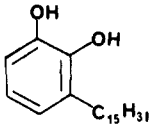
The *trans*-cyclohexanediols could also be transformed in vivo into electrophiles: epoxide or diketone (Scheme IV). In order to eliminate the epoxide formation hypothesis, *cis,trans*-3-pentadecyl-1,2-cyclohexanediol was prepared and used to sensitize a group of guinea pigs. The diol turned out to be a weak sensitizer and interestingly no cross-reaction was observed between the two groups of trans,trans and cis,trans sensitized guinea pigs (Figure 3). This excludes the possibility of a common metabolite (such as 3-pentadecyl-1,2-cyclohexanedione). At the same time, the epoxide route (impossible to be generated from a *cis*-diol) also seems excluded.



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Finally, the most important result from this study are probably the finding that *nonelectrophilic*, lipophilic derivatives are capable of inducing allergic contact dermatitis without the requirement of covalent bond formation. This seems to be the first true example of such a mechanism. The importance of lipophilicity in ACD mechanism has received confirmation at the immunological and cellular levels.[26,27]

Experimental Section

General Methods. Proton NMR spectra were recorded on a Brucker 200-MHz spectrometer in $CDCl_3$ unless otherwise specified. ^{13}C NMR spectra were taken on the same spectrometer at 50 MHz. Chemical shifts are reported in δ with respect to $CHCl_3$ as internal standard. Coupling constants (J) are expressed in hertz. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet). Infrared spectra were obtained on a Beckman Acculab spectrometer using $CHCl_3$ solutions; peaks are reported in reciprocal centimeters. Melting points were determined on a Büchi Tottoli 510 apparatus and are uncorrected. GLC analyses were performed with a Girdel 300 gas chromatograph with a 5% OV-17 column.

Dry solvents were freshly distilled before use. Tetrahydrofuran (THF) was distilled from sodium benzophenone. Triethylamine and hexamethylphosphoramide (HMPA) were distilled from powdered calcium hydride: all air- or moisture-sensitive reactions were conducted in flame-dried glassware under an atmosphere of dry argon.

General Procedure for the Preparation of 3-Alkyl-1-[(trimethylsilyl)oxy]-1-cyclohexene. Method A. To a cold (−40 °C) suspension of copper(I) iodide (14.3 g, 75 mmol) in dry THF (200 mL) was added RLi (150 mmol) in THF or Et_2O. The resulting solution was stirred at −40 °C for 10 min, then cooled to −50 °C, and treated with 2-cyclohexen-1-one (4.81 mL, 50 mmol). After being stirred for 1 h at −40 to −50 °C, the mixture was cooled to −60 °C, and HMPA (8.74 mL, 50 mmol), triethylamine (27.9 mL, 200 mmol), and chloromethylsilane (25.4 mL, 200 mmol) were added. The mixture was allowed to warm to room temperature for over 1 h. The reaction was poured into a saturated aqueous NH_4Cl solution (200 mL) and extracted with hexane (3 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated under vacuum.

Method B. A stirred solution of alkylmagnesium bromide (73 mmol) in THF (200 mL) was treated with copper(I) iodide (1.43 g, 7.5 mmol) in one portion at room temperature and immediately cooled to −40 °C. The resulting mixture was treated with 2-cyclohexen-1-one (4.81 mL, 50 mmol). After being stirred for 2 h at −40 to −50 °C, the resulting enolate was quenched by consecutive addition of HMPA (8.74 mL, 50 mmol), Et_3N (13.93 mL, 100 mmol), and chlorotrimethylsilane (12.69 mL, 100 mmol). The mixture was then processed as described for method A.

***trans,trans*-3-Alkyl-1,2-cyclohexanediols 3.** To a solution of BH_3–Me_2S (Aldrich) (150 mmol) in THF (75 mL, 2 M solution) was added dropwise crude 3-alkyl-1-[(trimethylsilyl)oxy]cyclohexene (50 mmol) for 30 min. The mixture was stirred for 48 h, after which BH_3–Me_2S in excess was decomposed by slow addition of EtOH (50 mL). A 3 N NaOH aqueous solution (15 mL) was added, followed by dropwise addition of 30% H_2O_2 (15 mL), and the reaction mixture stirred for 2 h at room temperature. Potassium carbonate was then added to saturation of the solution, the organic layer was separated, and aqueous layers were extracted several times with AcOEt. Combined organic layers were dried over $MgSO_4$ and concentrated by rotary evaporation to leave crude diol. Purification by "flash" chromatography gave pure 3-alkyl-1,2-cyclohexanediol.

***cis,trans*-3-Alkyl-1,2-cyclohexanediols 5.** A stirred solution of alkylmagnesium bromide (75 mmol) in THF (200 mL) was treated with copper(I) iodide (1.43 g, 7.5 mmol) in one portion at room temperature and immediately cooled to −40 °C. The resulting mixture was treated with *cis*-2,3-epoxycyclohexanol[20,21] (3.42 g, 30 mmol). After being stirred for 2 h at −40 °C, the mixture was allowed to warm to room temperature and stirred overnight in saturated aqueous NH_4Cl solution (200 mL) and extracted with hexane (3 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated under vacuum. The crude *cis*-diol (50 mmol) was treated overnight at room temperature with an excess of 2,2-dimethoxypropane (12.3 mL, 100 mmol) in acetone (100 mL) with a catalytic amount of *p*-TsOH. The resulting mixture was concentrated under vacuum and acetonide separated from **6** by flash chromatography (hexane/AcOEt, 9:1). The crude acetonide was treated with MeOH (100 mL) and *p*-toluenesulfonic acid (catalytic amount) to release diol **5**. After concentration under vacuum, purification by flash chromatography gave pure *cis,trans*-3-alkyl-1,2-cyclohexanediol **5**.

***cis,trans*-3-Pentadecyl-1,2-cyclohexanediol (5)**: white crystals; mp 68 °C; IR ($CHCl_3$) 3580–3440 ($\bar{\nu}$ OH); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 6.4 Hz), 1.26 (m, 29 H), 1.41–1.86 (m, 6 H), 3.28 (dd, 1 H, J_{23} = 9.1 Hz, $J_{1'2}$ = 3.0 Hz), 3.94 (ddd, 1 H, J_{23} = 9.1 Hz, $J_{1'6}$ ~ 2.8, $J_{1'6'}$ ~ 5.2 Hz); ^{13}C NMR ($CDCl_3$) δ 75.68, 69.80, 38.38, 31.94, 30.99, 30.04, 29.70 (8C), 29.37 (2C), 28.77, 26.73, 22.69, 19.23, 14.10. Anal. ($C_{21}H_{42}O_2$) C, H.

2-*n*-Pentadecyl-1,3-cyclohexanediol (6): IR ($CHCl_3$) 3565–3420; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 6.4 Hz), 1.25 (m, 29 H), 1.46–1.78 (m, 2 H), 1.83–1.86 (m, 4 H), 3.73 (ddd, 2 H, J_{12} = J_{16} = 9.3 Hz, $J_{16'}$ = 3.4 Hz). Anal. ($C_{21}H_{42}O_2$) C, H.

3-Methyl-1,2-cyclohexanediol (3a). Method A: white crystals;[15] mp 40 °C; IR ($CHCl_3$) 3610–3440; ^1H NMR ($CDCl_3$) δ 1.02 (m, 1 H), 1.03 (d, 3 H, J = 6.4 Hz), 1.31 (m, 3 H), 1.67 (d, 2 H, J = 7.7 Hz), 1.96 (m, 1 H), 2.45 (s, 2 H), 2.95 (t-like dd, 1 H, J_{12} = J_{23} = 9.3 Hz), 341 (ddd, 1 H, J_{12} = 9.3 Hz, J_{16} = 10.3 Hz, $J_{16'}$ = 4.6 Hz); ^{13}C NMR ($CDCl_3$) δ 81.52, 75.38, 37.72, 33.40, 33.03, 23.45, 18.11.

3-Pentyl-1,2-cyclohexanediol (3b). Method A: white crystals; mp 66 °C; IR ($CHCl_3$) 3600–3440; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 6.6 Hz), 1.27 (m, 9 H), 1.7 (m, 3 H), 1.95 (m, 2 H), 2.08 (m, 1 H), 3.00 (dd, appearing like a triplet, 1 H, J_{12} = J_{23} = 9.1 Hz), 3.38 (ddd, 1 H, J_{12} = 9.1 Hz, J_{16} = 10.2 Hz, $J_{16'}$ = 4.6 Hz); ^{13}C NMR ($CDCl_3$) δ 79.69, 75.48, 42.59, 32.89, 32.23, 31.88, 29.96, 26.28, 23.41, 22.64, 14.00. Anal. ($C_{11}H_{22}O_2$) C, H.

3-Decyl-1,2-cyclohexanediol (3c): white crystals; mp 61 °C; IR ($CHCl_3$) 3590–3420; ^1H NMR ($CDCl_3$) δ 0.88 (*t*, 3 H, J = 6.5 Hz), 1.26 (m, 19 H), 1.74 (m, 3 H), 1.97 (m, 2 H), 2.15 (m, 1 H), 3.02 (t-like dd, 1 H, J_{12} = J_{23} = 9.3 Hz), 3.38 (ddd, 1 H, J_{12} = 9.3 Hz, J_{16} = 10.2 Hz, $J_{16'}$ = 4.6 Hz); ^{13}C NMR ($CDCl_3$) δ 79.82, 75.55, 42.60, 32.86, 31.93, 30.07, 29.96, 29.67 (3C), 29.34 (2C), 26.28, 23.41, 22.67, 14.09. Anal. ($C_{16}H_{32}O_2$) C, H.

***trans,trans*-3-Pentadecyl-1,2-cyclohexanediol (3d)**: white crystals; mp 72.5 °C; IR ($CHCl_3$) 3600–3420; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 6.5 Hz), 1.26 (m, 29 H), 1.75 (m, 3 H), 1.95 (m, 2 H), 2.14 (m, 1 H), 3.01 (t-like dd, 1 H, J_{12} = J_{23} = 9.3 Hz), 3.88 (ddd, 1 H, J_{12} = 9.3 Hz, J_{16} = 10.1 Hz, $J_{16'}$ = 4.6 Hz); ^{13}C NMR ($CDCl_3$) δ 79.81, 75.06, 42.57, 32.89, 31.76, 30.05, 30.02, 29.67 (7C), 29.65, 29.32 (2C), 26.30, 23.43, 22.65, 14.10. Anal. ($C_{21}H_{42}O_2$) C, H.

Biological Assays. Albino Himalayan spotted Füllingsdorf (from Hoffman La Roche, Basel) female guinea pigs weighing from

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300 to 500 g were sensitized as described by Klecak.[28] 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, three injections, five after boost). The sensitizing molarity used was 0.12 M (FCA/saline (1:1)). After 15 days rest, the elicitation was conducted by an open epicutaneous test (OEt): 25 μL of an ethanol solution of diol (see Table II for amount of diols) was deposited on the shaved flank of the animal (on a 2-cm² surface using a standard circular stamp). Tests were read at the 48th 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). Concentrations up to 5% in ethanol of cyclohexanediols were *nontoxic*. Control groups of eight animals (FCA treated) were used in each experiment.

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Notes

Pyrazolo[4,5-*c*]quinolines. 2. Synthesis and Specific Inhibition of Benzodiazepine Receptor Binding

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A series of 1-aryl-3,5-dimethyl-4,5-dihydro-1*H*-pyrazolo[4,5-c]quinolin-4-ones (**2a–e**) and 1-aryl-3-methyl-1*H*-pyrazolo[4,5-*c*]quinolines (**3–7a–e**) bearing different substituents at position 4 were prepared and tested for their ability to displace specific [³H]flunitrazepam binding from bovine brain membranes. The 5-*N*-methyl derivatives **2a–c,e** were the compounds that bound with the highest affinity within this class. The replacement of the carbonyl group with other substituents and the resulting aromatization of the pyridine moiety greatly decreased the binding affinity. From a Lineweaver–Burk analysis on the most active compound **2b**, it appears that the inhibition is a competitive one.

Since the reports of Squires and Braestrup[1] and Mohler and Okada[2] on the high affinity binding sites for benzodiazepines in rat brain tissues, a number of synthetic compounds with different structures have been found to possess high affinity for the benzodiazepine receptor either as agonists or as antagonists.[3]

Non-benzodiazepine compounds with affinity for the benzodiazepine receptor could be of potential importance as tools for studying the receptor itself and eventually for the introduction into clinical use of new classes of compounds having the same properties of benzodiazepines.

Cain et al.[4] in a study on β-carbolines have reported some of the requirements that affect the affinity for the receptor. They stated that a planar heteroaromatic system containing at least one nitrogen atom is necessary and that a carbonyl group adjacent to the nitrogen atom greatly augments the binding to the receptor.

Guzman et al.[5] recently verified the same requirements in other non-benzodiazepine compounds like canthines, isoquinolines, and imidazoquinolines. In the latter a phenyl substituent provides the necessary hydrophobicity for a better fitting.

Following these findings we have prepared some pyrazoloquinolin-4-ones bearing an aryl substituent at position 1 or at position 2.[6] A preliminary binding study on bovine brain membranes has shown that only the 1-aryl-3-methyl-4,5-dihydro-1*H*-pyrazolo[4,5-*c*]quinolin-4-ones possess activity in displacing specific [³H]flunitrazepam from its receptor site.[6]

In order to establish structure–activity relationships for benzodiazepine receptor binding in this class of compounds, we are now reporting the synthesis and binding properties of some new 1-arylpyrazolo[4,5-*c*]quinoline derivatives.

Chemistry. 1-Phenyl-3-methyl-4,5-dihydro-1*H*-pyrazolo[4,5-*c*]quinolin-4-one (**1a**), already reported in the literature,[7] was synthesized to compare its activity with that of the 1-aryl-3-methyl-4,5-dihydro-1*H*-pyrazolo[4,5-*c*]quinolin-4-ones (**1b–e**) previously reported by us.[6]

Compounds **1a–e** were reacted with methyl iodide to obtain the 5-methyl derivatives **2a–e**.

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