Citrated human PRP from normal, healthy donors, who had denied receiving medication for 10 days, was purchased from a commercial blood bank. The plasma was centrifuged at 164g to remove any remaining red blood cells and maintained at 25 °C until the experiments were performed. The reagents were added as 10-μL aliquots per 1 mL of PRP to give the specified concentration (see Pharmacology section).

Washed platelets were prepared by supplementing PRP with ethylenediaminetetraacetic acid (EDTA, 2.5 mM final concentration) and centrifuging the plasma at 800g for 10 min. The platelet-poor plasma was decanted, and the platelet pellet was resuspended in Ca^{2+}-free Tyrode buffer. Immediately prior to the aggregaton studies, the platelet suspension was supplemented with $CaCl_2$ (1 mM final concentration) and 0.1 mg/mL of fibrinogen.

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Registry No. 1, 71629-07-7; 2, 5288-67-5; 3, 85421-76-7; 4, 85421-77-8; 5, 2443-62-1; 6, 66-25-1; 7, 870-46-2; 8, 79201-37-9; 10, 79201-41-5; methyl hexanoate, 106-70-7; hydrazine, 302-01-2.

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Interaction of Conformationally Flexible Agonists with the Active Site of Sweet Taste. A Study of Arylureas

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The conformation of tolylureas has been studied by means of X-ray diffraction, NMR spectroscopy, and semiempirical quantum-mechanical calculations. The flat shape of meta and para isomers allows a good interaction with the model sites for bitter and sweet taste, respectively, whereas the ortho isomer cannot fit the sites because of the relative arrangements of the aryl and amide planes and because of poor hydrophobic interactions. The consistency of the conformational results with the sweet taste model site, previously proposed by the authors, is emphasized by the good fit of dulcine, a sweeter para-substituted arylurea.

Many attempts have been made in the last few years to give an interpretation of the sweet taste on the basis of the molecular structure of sweet compounds.[1-5] Besides its intrinsic importance for practical applications, this problem represents a unique opportunity in the field of quantitative structure–activity correlations, owing to the very large number of known compounds (of widely different chemical characteristics) that can impart this stimulus.[6-8] The first successful correlation among molecules as diverse as sugar, amino acids, saccharine, chloroform, nitroanilines, etc. was put forward by Shallenberger and Acree,[1] who identified an entity composed of two hydrogen-bonding groups, 2.5–3 Å apart, in all known sweet molecules. Such an entity, commonly referred as the AH–B entity, should bind to two complementary hydrogen-bonding groups located in the active site of the receptor protein. This hypothesis has been widely accepted and represents, in many cases, the only recognizable common feature among sweet tastants; however, it suffers from several notable exceptions and is certainly insufficient to explain quantitative differences in sweetening powers. To quote but one exception, it is very difficult to consider the aromatic CH group of *m*-nitroanilines[9] as a likely hydrogen bond donor.

In the last decade, several researchers have tried to identify other electronic features that, combined with the AH–B entity, could possibly account for quantitative differences in taste.

Some of these proposals refer uniquely to the possible importance of some functional groups,[4,5,10] e.g. CN, NO_2, CO_2^-, and NH_2, without any explicit reference to their spatial location with respect to the AH–B entity. Others refer to the importance of dispersion forces, often associated with the presence of aromatic rings in sweet tastants.[4,11,12]

A combination of these ideas with the need of taking into account a precise steric relationship of new subsites with respect to the AH–B entity is present in the popular model of Kier.[2] This author suggests the existence of a third subsite, hydrophobic in nature, at the apex of a triangle whose basis is formed by the AH–B entity. This third site has been identified, in turn, with an aromatic carbon atom, a double bond, etc. It is likely that all these hypotheses reflect some true feature of the active site, but they share a common weak point: all of them disregard completely two very important prerequisites, i.e., the importance of the three-dimensional shape of the agonists and the limitation imposed by the sheer (equilibrium) volume of the active site. It is trivial to note that a molecule possessing Kier's third site and an ideal AH–B entity will, notwithstanding, never elicit sweet taste if it is so large as not to enter the receptor molecule.

It is therefore necessary to identify first the main sterical features of the active site and then proceed to unravel new electronic features. An important step along this direction is represented, in our opinion, by the model we proposed a few years ago.[3] This model was built on the basis of the

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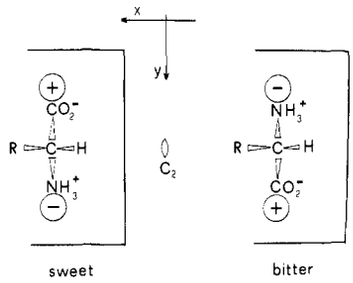

Figure 1. Scheme of the symmetry relationship between the AH–B entities of the active sites for sweet and bitter tastants. The C_2 axis interchanges only AH (+) and B (−).

peculiar relationship existing between several pairs of sweet and bitter isomers, notably simple amino acid enantiomers.[13] It was shown[3] that the simplest way of avoiding the paradox of two sites made up of two mirror-image proteins is to postulate, for the receptor sites of sweet and bitter molecules, two very similar flat cavities in which the hydrogen-bonding groups proposed by Shallenberger and Acree[1] for the sweet receptor (their AH–B entity) are interchanged (see Figure 1). The interactions of enantiomers with the two sites can only be very similar if the two cavities are nearly bidimensional: Figure 1 shows that the side chains (R) of the amino acid enantiomers are essentially outside the cavities. Accordingly, it becomes much simpler to find the contours of the active site in the plane containing the AH–B entity by using several substituted saccharines and aspartame as molecular molds[3a] for the sweet site and tetraiodosaccharine and D,D-aspartame for the bitter site.[3b]

The main features of our model can be summarized as follows. The principal "electronic feature" of the site is the AH–B entity of Shallenberger;[1,14] the overall shape of the site is that of a very flat hemihedral cavity, as indicated by the relationship between sweet and bitter enantiomers; the walls perpendicular to the main plane of the site are defined by the shapes of several substituted saccharines; a bulky apolar group at the far end of the plane, with respect to the AH–B entity, favors an increase in sweetness. Several drawings of this model are shown below in the description of the interaction of arylureas with the receptor site (vide infra). An important corollary of this proposal is that one side of the site (i.e., that perpendicular to the positive x direction of Figure 1) is completely open.[3] This circumstance may explain why even bulky substituents out of the main molecular plane have no influence on the taste of some sweet (or bitter) molecules. An extreme example is furnished by sweet macromolecules, either synthetic[15] or of natural origin.[16] This model proved quite satisfactory with many conformationally rigid[17] sweet and bitter compounds, some of which were not even quoted in ref 3a,b due to their similarity with the published examples. An independent test of the our model showed its consistency with the semiplanar forms of phyllodulcin and related compounds.[18]

On the other hand, there are several classes of sapidants that cannot be reconciled with our model on inspection, either because of their flexibility or because of the ambiguous nature of the AH–B entity or even because of the presence of electronic features not yet taken explicitly into account.

In all cases of conformationally flexible molecules, it is essential to perform a detailed conformational analysis before attempting any comparison with the model site.

The object of the present paper is an analysis of a class of flexible molecules, arylureas, whose taste varies widely as a function of isomerism.[7] Arylureas represent a suitable class of flexible tastants, since the analysis of their conformation can be restricted to only two internal rotation angles. We concentrated our study on the conformational analysis of the three isomeric tolylureas, owing to the striking dependence of taste on the position of the methyl group on the phenyl ring; it has been reported that *p*-tolylurea is sweetish, whereas the meta isomer is bitter and the ortho isomer is tasteless.[7] This sharp variation can afford a clearcut test of our model of active site, provided we are able to describe the conformational state of these molecules in a way that can be related to their interaction with the receptor site.

Conformational State of Arylureas. The conformation of arylureas, and in particular of the tolylureas that are the object of our study, are dominated by two internal rotation angles, i.e the dihedral angle of the amide bond and that around the *N*-aryl bond.

Different methods were used to determine the conformational preferences around these two bonds. A wealth of literature data[19] indicate that only two values of the rotation angle around the amide bond are accessible, i.e., 0 (cis) and 180° (trans), with a large preference for the latter. NMR spectroscopy methods have been widely used for these types of compounds, both to measure the residence times of the two isomers and to evaluate the barrier of isomerization.[19] ^1H and ^{13}C NMR spectra at various temperatures were run for all tolylureas, mainly in an attempt to evaluated the relative populations of cis and trans isomers. It is important to note that in order to observe the cis isomer one is confronted with opposite experimental requirements. Temperature changes, in fact, will influence both the populations of the isomers and the rate of interconversion; thus, an increase of temperature will favor the population of the cis isomer (less stable) but, at the same time, will make it much more difficult to measure, since it increases the rate of isomerization, possibly favoring the merging of resonance pairs into single peaks. When the expected values of the barriers (around 20 kcal/mol) and the maximum observed values for the populations of the cis isomer in simple *N*-substituted amides[19] (i.e., a few unit percent) are taken into account, it appears that one can restrict the useful temperature range to ca. ±30 °C around room temperature. Both ^1H and ^{13}C NMR spectra of the three ureas were recorded in CD_3OD solutions between −10 and +50 °C. All spectra consistently showed a number of resonances typical of a single isomer, with not even indirect indications of the presence of significant amounts of the second isomer (e.g., broadening of the lines). This finding was considered consistent with the literature data indicating a cis population below 1% for the (rather similar) corresponding acetanilides. Accordingly, only the trans conformer was taken into account in many of the subsequent conformational considerations. [Nevertheless, the possibility that the cis species (very

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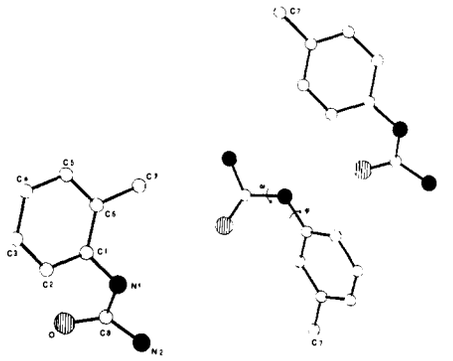

Figure 2. Molecular models of the tolylureas derived from solid-state studies. The numbering of the atoms is the same of Table I.

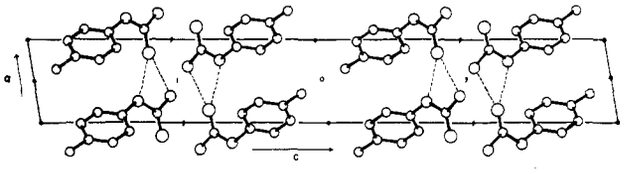

Figure 3. Projection of the molecular packing of *p*-tolylurea along the *a* axis. Only hydrogen bonds between molecules of the same height are indicated by the dotted lines.

dilute) might interact with the receptor site was explicitely considered (vide infra).]

The rotation around the *N*-aryl bond is even more difficult to study by means of direct physicochemical methods, because of the low value of the barrier and the presumably small differences among the limiting (minimum energy) conformations. We decided to resort to internal energy calculations, combined with X-ray diffraction studies. That is, we have determined the crystal structure of the three tolylureas[20,21] with the goal of obtaining good molecular parameters for the subsequent internal energy calculations. Obvious but important "side results" of these determinations are the minimum energy conformations in the solid state. These conformations, although not representative of the conformational state in solution (nor of the state that interacts with the receptor), are useful reference structures for a comparison with the results of the conformational analysis. Figure 2 shows the molecular models derived from the crystal-structure analyses of the three isomers. The three structures are very similar; in particular, all three have trans amide bonds and torsion angles around the *N*-aryl bonds on the order of 50°. This value can be considered as a compromise between the need of optimizing delocalization (that would require completely flat, highly energetic conformations) and the stringent limitations imposed by the network of intermolecular hydrogen bonds present in the molecular packings. Figure 3 shows the projection of the structure of *p*-tolylurea along the *b* axis. It can be seen that the tolyl rings are in close contact, and, accordingly, any torsion angle smaller than 50° would require a substantial weakening of the hydrogen bonds. Thus, it can be anticipated that isolated tolylureas may have conformations with smaller values of this angle, even if not completely planar, as shown by energy calculations.

Both empirical and semiempirical methods can be used for a molecule of the size of tolylurea. Semiempirical

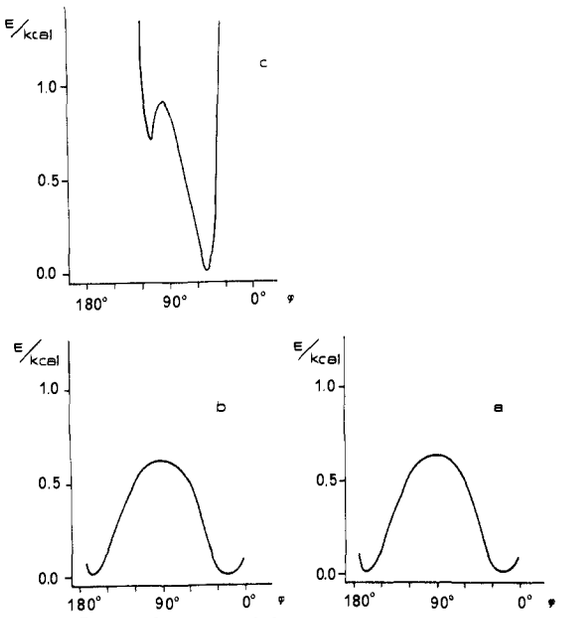

Figure 4. Internal energy of the trans conformers of tolylureas as a function of ϕ calculated by means of nonbonded methods. Graphs *a* and *b* refer to the para and meta isomers, respectively. Owing to the distance of the methyl group from the amide moiety, graph *b* is nearly indistinguishable from graph *a*. The absolute minima are at 20°. The minimum for the ortho isomer is at 45°.

methods are certainly more suited to treat cases in which delocalization plays an important role. Accordingly, we decided to employ PCILO methods,[22] whereas empirical calculations were done mainly for a comparison. Solid-state results hint to purely trigonal nitrogen atoms; notwithstanding, a slightly pyramidized nitrogen was used in the energy calculations, since geometry optimization through MNDO methods[23] do indicate a moderate distortion from planarity.

The most prominent feature of the PCILO calculations is that all three isomers show very similar minimum energy calculations (see paragraph at the end of paper concerning supplementary material). These conformations have trans amide bonds and a torsion angle for the *N*-aryl bond around 30°, i.e., a value significantly lower than those found in the solid state, that leads to rather flat overall shapes for these molecules.

However, the great similarity among the calculated conformations may reflect, in part, an overstimation of resonance energy, a pitfall of some PCILO calculations. Accordingly, we have also calculated the energy profiles for torsion around the *N*-aryl bond with empirical methods, thus deliberately "underestimating" resonance effects.

As described under Experimental Section, we adopted a simple 6–12 potential with the parameters suggested by Harmony et al.[24] The results are shown in Figure 4. It is interesting to see that even a complete neglect of delocalization leads to flat molecular shapes. Furthermore, it may be worth noting that these calculations differentiate the ortho isomer with respect to its meta and para counterparts.

Interaction with the Site. A comparative evaluation of PCILO and empirical calculations tells us that the most populated conformation in solution for the para and meta

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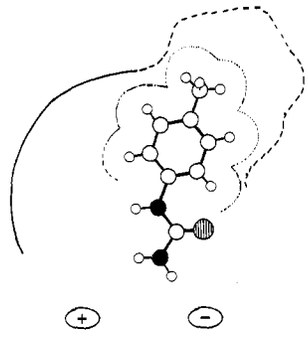

Figure 5. Fit of the minimum energy conformation of *p*-tolylurea in the model site for sweet taste. The aryl group interacts very well with the hydrophobic part of the active site.

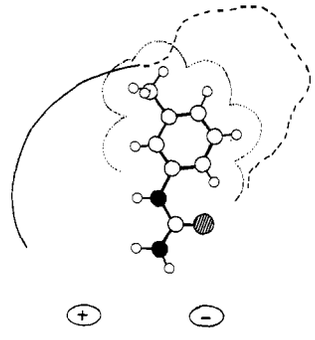

Figure 6. Interaction of the minimum energy conformation of *m*-tolylurea with the model site for the sweet taste. The methyl group *invades* one of the walls.

isomers is characterized by a trans amide bond and a torsion angle around the *N*-aryl bond of 20–30°, since the profiles of Figure 4 show minima at ca. ±20° separated by a very small barrier, whereas the PCILO maps (see paragraph at the end of paper concerning supplementary material) show minima at 30° with substantially higher barriers. Similar considerations point to a lower flexibility around the *N*-aryl bond for the ortho isomer with a sharper minimum in the neighborhood of 45°.

Conformations with a cis amide bond would be equally consistent with the site but can be ignored, since both experimental results and calculations show that their concentration is one or two orders of magnitude smaller than that of the corresponding trans isomers.

Trans conformations, however, can interact with the model site only if we relax the criterion that concerns the AH–B entity, i.e., if we use only one of the hydrogen-bonding groups of the molecule, a situation never taken into account by Shallenberger or Kier but already discussed by us in other cases. In fact, it appears quite permissible to consider the AH–B entity and the shape of the molecule as equally important in determining the fit. Thus, we can have molecules that can bind strongly to the receptor only through the two hydrogen bonds of the AH–B entity, without "filling" the volume of the site (e.g., some simple amino acids), and, conversely, molecules that form only one hydrogen bond, which fit the shape of the site very well (e.g. *m*-amino-*p*-alkoxynitrobenzenes[3]). Figure 5 shows that, indeed, this is the case for *p*-tolylurea; it forms one hydrogen bond with the unsubstituted amino group, and the aromatic part adapts very well to the upper part of the model site previously indicated as responsible for strong hydrophobic interactions. It must be noted that this arrangement places the CO group in a position very similar to the positions of the CO groups of saccharine, dioxolanes, and aspartame.[3] It seems fair to propose this position for a soft base as indicative of a secondary electronic feature. Figure 6 shows that the meta isomer cannot enter the sweet receptor without invading one of the walls, but it can adapt very well to the (larger) shape of the bitter taste receptor site[3b] (Figure 7). It is worth noting that in this case we can make full use of the AH–B entity, a circumstance that improves the interaction with the bitter receptor.

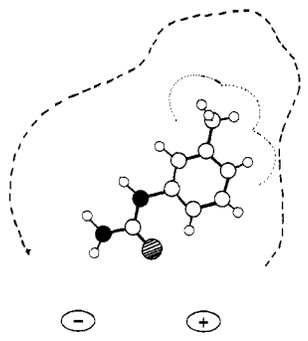

Figure 7. Fit of the minimum energy conformation of *m*-tolylurea with the model site for the bitter taste.

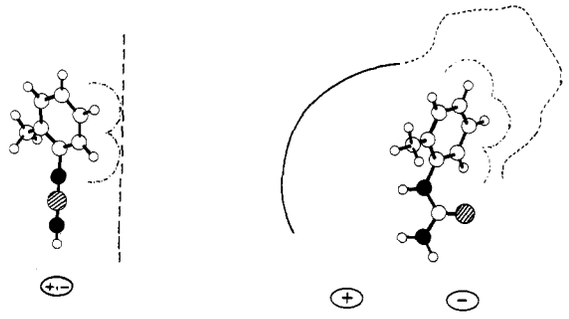

Figure 8. Interaction of the minimum energy conformation of *o*-tolylurea with the model site for the sweet taste. Although the aryl ring does not invade the Shallenberger barrier, the interaction with the hydrophobic plane of the site is very poor.

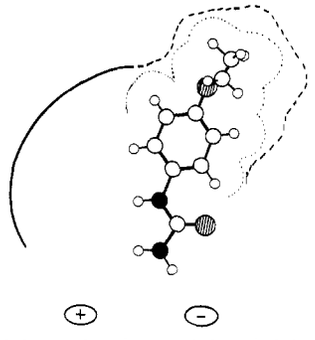

Figure 9. Fit of the molecular model of dulcine (corresponding to the conformation of *p*-tolylurea) in the model site for the sweet taste.

The ortho isomer, on the other hand, can fit neither model site, not only because of the slightly different angle of the aryl ring but also because of the absence of any close interaction with the walls in the hydrophobic part of the site (Figure 8).

Indeed, only completely flat conformations of the arylureas could have an optimum interaction with our model sites. Accordingly, even the comparatively small increase of the ϕ torsion angle for the ortho isomer may be sufficient to prevent a successful fit. In the case of the meta and para isomers, on the other hand, the fit is strengthened by close hydrophobic interactions with the walls of the sites. That this is indeed the case is substantiated by a comparison between the fits of the para isomer (sweetish) and of the

Table I. Relevant Molecular Parameters of the Three Isomers of Tolylurea, as Used in the Energy Calculations

	ortho	meta	para
N2-C(O)	1.341 Å	1.332 Å	1.332 Å
N1-C(O)	1.358 Å	1.353 Å	1.353 Å
C(O)-O	1.246 Å	1.245 Å	1.245 Å
N1-C1	1.426 Å	1.424 Å	1.423 Å
N1-C(O)-N2	115.3°	115.4°	115.4°
N2-C(O)-O	122.0°	121.6°	122.0°
C(O)-N1-C1	124.5°	124.5°	124.7°
C4-C7			
C5-C7		1.492 Å	
C6-C7	1.495 Å		1.499 Å
C3-C4-C7			
C4-C5-C7		120.6°	
C5-C6-C7	120.9°		120.2°
N2-H1(N2)	1.0 Å		
N2-H2(N2)	1.0 Å		
N1-N(N2)	1.0 Å		
H1-N2-C(O)	119.1°		
H2-N2-C(O)	122.2°		
H(N1)-N1-C1	119.2°		
C-C[a]	1.39 Å		
C-H	1.0 Å		
C-C-C	120.0°		
C-C-H	120.0°		
C-H[b]	1.08 Å		
C-C-H	109.5°		

[a] Phenyl ring parameters. [b] Methyl parameters.

dulcine molecule (very sweet). Figure 9 shows that a bulkier group in the para position does indeed allow a better fit into our model site of this arylurea, with respect to tolylureas, compensating for the lack of the B part of the AH–B entity. The molecular conformation of dulcine was calculated by means of PCILO methods and is coincident with that of the para isomer for the common moiety.

Conclusion

Our results lead to the conclusion that the models previously proposed for the active sites of the receptors of the sweet[3a] and bitter[3b] molecules can be used to explain the taste of conformationally flexible molecules, at least in cases of moderate complexity, such as that of tolylureas. It seems interesting to emphasize that, although this study required a fairly complex and time-consuming conformational analysis, its results can be easily extended to other arylureas. Thus, it is almost obvious to observe that the taste of *p*-methoxyphenylurea, reported as very sweet,[7] and that of *o*-methoxy- and *o*-ethoxyphenylureas, both reported as tasteless,[7] are consistent with the requirements of our models. In our view, all these findings strengthen the belief that the simple geometrical models proposed[3] for the receptor sites can form the basis for sound molecular theories of sweet and bitter tastes and, consequently, for efficient drug design in the field of synthetic sweeteners.

Experimental Section

Materials. All three tolylureas were prepared according to a standard procedure described by Vogel,[25] by reacting the parent toluidine with urea in a solution containing hydrochloric acid and acetic acid. Purification was achieved by two recrystallizations from ethanol–water mixtures. Crystals suitable for X-ray diffraction analysis were obtained by means of slow crystallization from 1:1 (v/v) mixtures of ethanol and benzene. NMR samples were prepared with methanol-d_4 (C. Erba, Milano) as solvent and Me_4Si (C. Erba, Milano) as internal reference. Concentrations of 1.0 (w/v) and 5.0% (w/v) were employed for ^1H and ^{13}C NMR, respectively.

Methods. X-ray Analysis. The following crystal data were collected for the three isomers of molecular formula $C_8H_{10}N_2O$, M_r 150.181: para, space group $P2_1/c$, a = 4.628 (1), b = 5.449 (1), c = 31.991 (2) Å, β = 99.66 (8)°, U = 796.39 Å^3, D_m = 1.24 g cm^{-3}, D_c = 1.252 g cm^{-3} for Z = 4, Cu $K\alpha$, λ = 1.5418 Å, 1160 independent reflections; meta, space group $Pccn$, a = 21.095 (3), b = 8.539 (1), c = 9.014 (3) Å, U = 1623.69 Å^3, D_m = 1.23 g cm^{-3}, D_c = 1.239 g cm^{-3} for Z = 8, Cu $K\alpha$, λ = 1.5418 Å, 1592 independent reflections; ortho, space group $P2_1/c$, a = 4.652 (6), b = 5.999 (2), c = 27.55 (2) Å, U = 766 Å^3, D_m = 1.28 g cm^{-3}, D_c = 1.30 g cm^{-3} for Z = 4, Cu $K\alpha$, λ = 1.5418 Å, 1137 independent reflections.

Intensity data were collected on a four-circle automated diffractometer (CAD-4 ENRAF-NONIUS), equipped with a PDP-11 computer. All three structures were solved by weighted multisolutions tangent refinement[26] with the program MULTAN included in the PDP-11 structure determination package (SDP). Several refinement cicles led to the following R values (including hydrogen atoms): 0.052, 0.066, and 0.052 for para, meta, and ortho isomers, respectively.

Further details on the structure determinations have been published elsewhere.[20,21] The relevant molecular parameters used in energy calculations are reported in Table I with reference to the numberings of Figure 1.

NMR Measurements. ^1H and ^{13}C NMR spectra were recorded on a Bruker WH-270 spectrometer, equipped with a variable-temperature accessory, in the range 260 to 320 K. Memory blocks of 8K and 16K were used for spectra accumulation and memory blocks of 16K and 32K were used for FT in the case of protons and carbons, respectively.

Energy Calculations. Energy calculations were extensively employed in this paper in the conformational analysis of the tolylureas. Quantum mechanical methods were predominant, whereas empirical methods were used only for comparison.

As discussed in a previous section, the angle that requires accurate energy calculations is the torsion angle around the *N*-aryl bond (ϕ), whereas the conformational state around the amide N–C′ bond (ω) can be defined (satisfactorily) by means of spectroscopic methods. Notwithstanding, we performed a complete quantum mechanical calculation of the (ω, ϕ) potential energy surface.

A complete analysis of the large potential energy surface of molecules containing 11 second period atoms performed with ab initio methods was considered exceedingly expensive even at the minimal basis set level.[27] Accordingly, we resorted to semiempirical methods. Among the semiempirical methods using the

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NDO approximation,[28] PCILO[22] proved quite reliable for molecules containing conjugated systems,[29] even if comprising heteroatoms.[30] The version of PCILO actually used, in our calculations was the CNDO/2 as written for program no. 220 of the Quantum Chemistry Program Exchange (QCPE, Indiana University, Bloomington, Indiana). Care was taken, in the course of the calculation of the (ω, ϕ) surface, to optimize bond polarities, since the use of constant polarities, although greatly speeding the calculations, may give rise to an unusually high spurious barrier across local minima.[31]

Bond distances and valence angles involving heavy atoms were taken directly from the X-ray analysis,[20,21] except for the aromatic rings.[32] C–H bonds were taken all equal to 1.00 Å for the aromatic rings[32] and to 1.08 Å for the methyl groups.[32] Bond distance and valence angles of hydrogens bound to nitrogens were optimized by using the MNDO[23] approximation and the X-ray data for the remaining geometrical parameters. The results show a slight pyramidalization of the nitrogens, whereas bond distances are in good agreement with a neutron diffraction study on urea.[33] The pukering of the nitrogen destroys the symmetry of the (E, ϕ) potential around $\phi = 0°$ or $\phi = 180°$, although nitrogen inversion is likely coupled to the ϕ torsion.

On the other hand, preliminary (ω, ϕ) potential energy surface scans with flat nitrogen atoms showed only minor differences with respect to the more sophisticated final maps. Empirical calculations were performed by means of a general program developed by us.[34] The only potential employed in these calculations was a Lennard-Jones 6–12 potential with the parameters proposed by Harmony et al.[24]

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Registry No. *p*-Tolylurea, 622-51-5; *m*-tolylurea, 63-99-0; *o*-tolylurea, 614-77-7.

Supplementary Material Available: Energy maps (Figures 10–12) of *p*-, *m*-, and *o*-tolylurea as a function of the torsion angles ω and ϕ, calculated by the PCILO method (3 pages). Ordering information is given on any current masthead page.

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Cloxacepride and Related Compounds: A New Series of Orally Active Antiallergic Compounds

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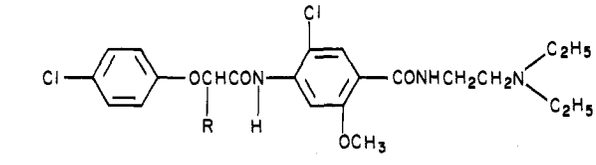
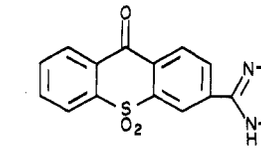
4-[[(*p*-Chlorophenoxy)acetyl]amino]-5-chloro-2-methoxy-*N*-[2-(diethylamino)ethyl]benzamide (cloxacepride, 1), exhibited substantial oral antiallergic potential in a reaginic PCA test in rats over a wide range of antigenic challenge times. Available reference compounds with oral activity, such as doxantrazole and 7-(2-hydroxyethoxy)-9-oxoxanthene-2-carboxylic acid (AH 7725, 4), were active only when administered 15 min before challenge: 4, in particular, was not consistent in effect. Oral ED_{50} values for cloxacepride of 46–49 mg/kg were comparable to that of theophylline and to an intravenous injection of 2 mg/kg of disodium chromoglycate (DSCG) followed by immediate challenge. Following oral ED_{50} doses, 1 showed slower onset and longer duration of action than theophylline. The absence of inhibition of systemic anaphylaxis and of antihistaminic activity suggests specific effect for reaginic antigen antibody reactions. Structure–activity relationships of various chemical modifications were investigated and discussed in terms of essential substituents.

Since the discovery of cromolyn sodium (DSCG)[1] as an inhibitor of mediator release in sensitized tissues, a new antiallergic research area was created to find new DSCG-like but orally active compounds. In addition to DSCG-related chromone derivatives,[2,3] some structurally different compounds were found,[4–6] e.g., doxantrazole,[7] AH 7725 (4),[8] bufrolin,[9] WY-16,922,[10] and M & B 22948.[11] The finding that DSCG, doxantrazole, and bufrolin inhibited the anaphylactic-type reaction but not the ionophore-induced release of histamine[12] supports the recent hypothesis that antiallergic drugs act by blocking the antigen-induced transport of calcium, i.e., by control of opening and closure of calcium gates in the mast cell membrane.[13]

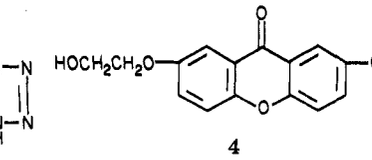
Screening a large series of various amides of metoclopramide (5) revealed that cloxacepride (1) and its methyl analogue 2 possess unique and unusual antiallergic potential. Neither metoclopramide nor different amides



1, R = H
2, R = CH_3



3



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bearing alkyl and/or aryl groups instead of the chlorophenoxy group showed any antiallergic efficacy. Further

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