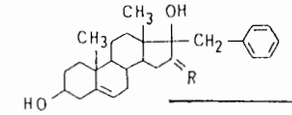
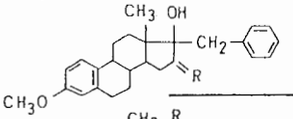
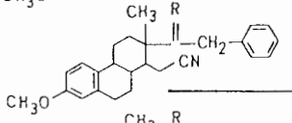
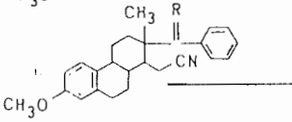
04-Crystallography of Biological Small Molecules

Molecular mechanic calculations were performed to provide and to determine minimum energy conformations. Computer aided modeling of the new structures with extended 17-side-chains has been attempted.

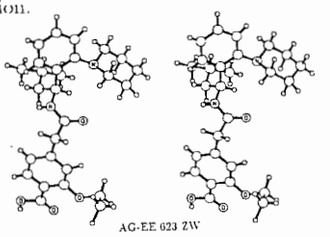
	COMPOUND	R	S.G.	Z	R
	1		$P2_12_12_1$	8	0.051
	2	=NOH	$P2_12_12_1$	4	0.058
	3	=O	$P2_1$	2	0.071
	4	=NOH	P1	2	0.069
	5	=O	$P2_12_12_1$	4	0.065
	6	=O	$P2_12_12_1$	4	0.048
	7		$P2_12_12_1$	4	0.069
	8	=O	$P2_1$	2	0.047
	9		$P2_1$	2	0.041

MS–0 .0 .08 X-RAY ANALYSIS OF THE NEW HYPOGLYCEM DRUG REPAGLINIDE: COMMON STRUCTURAL FEA1 RES WITH GLIBENCLAMIDE. By W. Grell, Department of Chemical Research, and M. Mark, Department of Biochemical Research, Dr. K. Thomae GmbH. Biberach, and P. Luger*, Institute for Crystallography, Freie Universität Berlin. Germany.

Repaglinide, (+)-2-ethoxy-α-[[(S)-α-isobutyl-o- piperidinobenzyl]carbamoyl]-p-toluic acid (1), AG-EE 623 ZW, (WHO Drug Inform. 1992, 6(3), List 32), being the most active representative of a series of novel hypoglycemic (B-cytotropic) benzoic acid derivatives, was found to display a 20 times higher blood sugar lowering activity than the sulfonylurea (s.u.) compound glibenclamide (2) in fasted rats. With the aim of obtaining more insight into common or different conformational aspects, the X-ray structure of (1) and of several related compounds was determined. The structure of (1) was computergraphically compared with that of (2) (Byrn,S.R.,McKenzie, A.T., Hassan,M.M.A. & Al-Badr,A.A., J. Pharm. Sci. 1986, 75, 596-600). The superposition was performed in such a way that functional groups which are known to contribute essentially to activity, and which are regarded as being involved in binding to the so called "s.u. receptor" (Kaubisch,N., Hammer,R.,Wollheim,C.,Renold, A.E. & Offord,R.E., Bioch. Pharmacol. 1982, 31, 1171-1174) fit best after having slightly rotated only a few bonds of each of the X-ray structures.

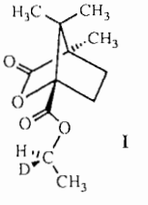
It was found that the acidic (-COOH / -SO2-NH-) and basic (piperidino -N / methoxy-O) functional groups, and the amino hydrogens (-H) are overlapping quite well. In contrast the amido oxo (=O) groups do not, but are yet in positions for enabling hydrogen bonds to the same (presumed) electron accepting binding site of the "s.u. receptor".

It is concluded that the conformations being realized in the superposition are compatible with a common three-point binding model. This, as an extension of the binding hypotheses discussed formerly (Rufer,C. & Losert,W., J. Med. Chem. 1979, 22, 750-752; Brown,G.R. & Foubister,A.J., J. Med. Chem. 1984, 27, 79-81), means that the three main functional groups [the acidic, the basic, and the (ambivalent) amido groups] bind simultaneously to corresponding binding sites of the "s.u. receptor". Additional lipophilic interactions are supposed to occur so that the particular groups of (1) [the (S)-positioned isobutyl group, the (whole) piperidino group, and the ethoxy group], and (2) [the cyclohexyl group], respectively, are binding into (presumed) different corresponding pockets of a given "s.u. receptor". This supports the speculation that "there exists more than one specific binding site for sulfonyl ureas and benzoic acid derivatives" (Verspohl, E.J., Ammon, H.P.T. & Mark, M., J. Pharm. Pharmocol. 1990, 42, 230-235). Possibly, even the existence of different (sub)types of "s.u. receptors" has to be taken into consideration. - Further investigations are necessary before coming to a decision between the various kinds of binding, and getting conclusive information about the "active conformations" of structurally different stimulators of insulin secretion.



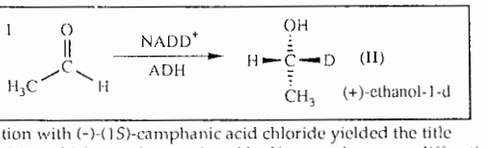
OPS–04.01.09 ABSOLUTE CONFIGURATION OF THE (-)-(1S)-CAMPHANATE ESTER OF (+)-ETHANOL-1-d BY NEUTRON DIFFRACTION by Robert Bau*, Tobias Metzenthin, Department of Chemistry, University of Southern California, Los Angeles, CA 90089, U.S.A., Thomas F. Koetzle, Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973, U.S.A., and Harry S. Mosher, Department of Chemistry, Stanford University, Stanford, CA 94305, U.S.A.

The absolute configuration of the (-)-(1*S*) camphanate ester (I) of (+)-ethanol-1-*d* has been determined by single-crystal neutron diffraction. (+)-Ethanol-1-*d* (II) was prepared (Scheme 1) via the reduction of acetaldehyde by the enzyme alcohol dehydrogenase (ADH) in the presence of deuterated nicotinamide adenine dinucleotide ($NADD^+$) by the method of Simon[1]:



I

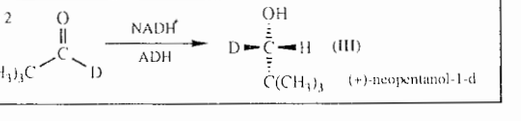
Scheme 1



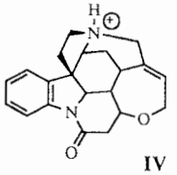
Esterification with (-)-(1S)-camphanic acid chloride yielded the title compound (I), which was then analyzed by X-ray and neutron diffraction. The neutron diffraction analysis of I, using the known[2] absolute configuration of the (-)-(1S) camphanate group as a reference, showed that the absolute configuration of the chiral CHD group is *R*.

We had earlier analyzed[3] the absolute configuration of (+)-neopentanol-1-*d* (III), prepared by the reduction of deuterated neopentanal by actively fermenting yeast[4] (presumably also involving alcohol dehydrogenase; Scheme 2) and converted to its phthalate half-ester (V):

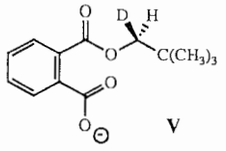
Scheme 2



Neutron diffraction analysis of the strychnine salt (IV) of (+)neopentyl-phthalate-1-*d* (V) then showed that the absolute configuration of the CHD group of V is S.[3]



IV



V

This pair of neutron diffraction analyses shows that (+)-ethanol-1-*d* (II) and (+)-neopentanol-1-*d* (III), prepared by complementary methods (Schemes 1 and 2, respectively), have opposite absolute configurations (*R* and *S*, respectively), as predicted,[4] despite the fact that their ORD rotations are of the same sign (+), in both cases).[5] This work shows unequivocally that alcohol dehydrogenase delivers hydrogen atoms to the *re* face of an aldehyde, and also confirms suspicions[4] that the optical rotatory properties of ethanol-1-*d* is different than those of its higher homologs.

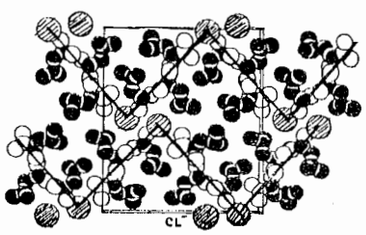
(+)(*R*)-Ethyl-1-*d* (-)(1*S*)-camphanate (I) crystallizes in the orthorhombic space group $P2_12_12$, with a = 6.422(1), b = 21.004(4), c = 9.275(2) A. Neutron data were collected at room temperature on a sample with dimensions 2.2 x 3.7 x 2.4 mm^3 at the Brookhaven High Flux Beam Reactor. The structure was refined to final agreement factors of $R(F^2) = 0.083$ and $R(wF^2) = 0.075$ for 1093 reflections with $F_o^2 > 1\sigma(F_o^2)$.

(1) (a) H. Guenther, F. Biller, M. Keilner and H. Simon, *Angew. Chem. Internat. Edit.*, **12**, 146-147 (1973)
(b) H. S. Mosher and C. W. Mosher, *Ind. J. Chem.*, 31, 900 (1992)
(2) W. Klyne and J. Buckingham, *Atlas of Stereochemistry*, 2nd. ed., Oxford University Press, London, Vol. 1, page 85 (1978).
(3) (a) Hanna S. H. Yuan, Ph. D. Dissertation, Univ. of Southern Calif. (1989)
(b) H.S.H. Yuan, R.C. Stevens, R. Bau and H.S. Mosher, manuscript in prep.
(4) C. Fisher, E. Morsè, B. Romer, T. P. You, C. W. Mosher and H. S. Mosher, *Tetrahedron*, 48, 2993-3000 (1992)
(5) A. Gedanken, C. W. Mosher and H. S. Mosher, *Chem. Commun.*, 430 (1992)

OPS–04.01.10 PEPTIDE MODELS FOR UNDERSTANDING PACKING INTERACTIONS IN THE INTERIOR OF GLOBULAR PROTEINS: CRYSTAL STRUCTURE OF LEUCYL-LEUCYL-LEUCINE HCL. By E. Subramanian*, S.S.Rajan and V.Jayashree, Department of Crystallography and Biophysics, University of Madras, Madras 600 025, INDIA.

The interior of globular proteins is characterized by tightly packed sidechains of hydrophobic residues. Prediction of protein folding requires an understanding of the patterns of packing of these sidechains. As a starting model for prediction, one can use the geometry of packing interactions as observed in the crystal structures of small peptides containing hydrophobic residues. A study of the pairwise interactions of the 20 different residues, as observed in the interior of several proteins whose crystal structures are known, reveals that leu...leu interactions are the most frequent. To understand the pattern of leu...leu interactions, the peptide leucyl-leucyl-leucine HCl has been crystallised. The crystals are orthorhombic, space group $P2_12_12_1$, with a=5.174(2), b=19.371(3), c=22.976(3)A and Z=4. CuKα diffractometer data ($\Theta \leq 60^0$) were used to solve the structure by direct methods, and to refine it to an R-index of 0.08. The C^δ atoms in all the sidechains display high thermal motion. The peptide units are trans. The peptide backbone assumes an extended parallel beta sheet conformation, with Ψ_1=128, ω_1=171, (ϕ_2, Ψ_2)=(-119,133), ω_2=179, (ϕ_3, Ψ_3)=(-85, 170). The three leucyl sidechains adopt the commonly observed and energetically favourable conformations (t,g^+), (g^-,t) and (g^-,t) respectively.

Crystal packing involves successive peptide molecules, related by the a-translation of the lattice, forming an infinitely extended ribbon of parallel beta pleated sheet structure, with one inter-chain hydrogen bond of length 3.05A between backbone amide and carbonyl groups. The leucyl sidechains stick out on both sides of the sheet. Symmetry-related molecules also lie in similar ribbons, and all such sheets are linked by 'hinges' formed of hydrogen bonds between Cl^{-1} ions on the one hand and NH_3^+ and -COOH terminals of the peptide molecules on the other. The net result is to produce a zig-zag 'head-to-tail' link up of the peptide molecules, effectively creating a super-pleated-sheet arrangement. The packing of such super-pleated sheets introduces a novel way of segregating the hydrophobic groups. The layers of polar groups are arranged in a zig-zag pleated fashion, reminiscent of 'corrugated' sheet structures. The polar 'ridges' in the 'corrugated' sheets face the polar 'grooves' of neighbouring sheets and vice versa. All the hydrophobic leucyl sidechains are effectively confined to the gaps between the corrugated sheets. Details of sidechain interactions will be presented.



PS–04.01.11 THE EFFECTS OF METAL-BINDING ON A NUCLEOBASE: A COMPARISON OF CHARGE DENSITY IN ADENINE HYDROCHLORIDE AND ITS ZINC COMPLEX. By L.M. Cunane and M.R. Taylor*, School of Physical Sciences, The Flinders University of South Australia, GPO Box 2100, Adelaide, S.A., 5001, Australia.

This study of charge density and electrostatic properties in adeninium hydrochloride hemi-hydrate and $1H^+$-adeniniumtrichlorozinc(II) is part of a broader investigation into the effects of substituents, protonation and bound metal ions on electron density distributions in some nucleobase derivatives.

High-resolution X-ray data sets to $\sin\theta/\lambda = 1.32$ $Å^{-1}$ were subjected to conventional and multipole refinement. These show that there are significant differences between the dimensions of the nucleobases in the two structures. The charge density was determined from refined pseudoatom models at the octapole level. Final difference maps had minima and maxima of -0.16 and +0.20 e $Å^{-3}$ for the hydrochloride and -0.29 and +0.38 e $Å^{-3}$ for the zinc complex.

Deformation density maps have been calculated, and details of the electron density distributions in the bonds have been deduced. Net atomic charges from the multipole refinements have reasonable values with N1, C4, C5 and C8 being more positive in the zinc complex than in the hydrochloride indicating the net withdrawal of electron density from the complexed nucleobase. In both compounds, C8 has a significant negative charge and the C8-H8 bond is the most polar of the C-H bonds in line with the acidic nature of this proton.

The electrostatic potential of the molecules, isolated from the crystal structure, have also been mapped. The lines of positive equipotential are much more compact in the hydrochloride, compared with its zinc complex, where the positive potential extends well out from the molecule. As a consequence the protons of the complexed nucleobase are shown to be more acidic than those of the uncomplexed one. The increased positive electrostatic potential for the nucleobase bound to zinc will enhance base-base hydrogen bonding.