(11) S. Archer, M. R. Bell, T. R. Lewis, J. W. Schulenberg, and M. J. Unser, *J. Amer. Chem. Soc.*, 80, 4677 (1958).
(12) S. Archer, T. R. Lewis, and B. Zenitz, *ibid.*, 80, 958 (1958).
(13) A. Albert and R. Royer, *J. Chem. Soc.*, 1148 (1949).
(14) C. L. Zirkle and C. Kaiser in "Medicinal Chemistry," 3rd ed, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, p 1410.
(15) J. J. H. McDowell, *Acta Crystallogr., Sect. B*, 25, 2175 (1965).
(16) A. S. Horn and S. H. Snyder, *Proc. Nat. Acad. Sci. U. S.*, 68, 2325 (1971).
(17) R. Bergin and D. Carlström, *Acta Crystallogr., Sect. B*, 24, 1506 (1968).
(18) B. Carlström and R. Bergin, *ibid.*, 23, 313 (1967).
(19) A. Carlsson and M. Lindqvist, *Acta Pharmacol. Toxicol.*, 20, 140 (1963).
(20) H. Nyback and S. Sedvall, *J. Pharmacol. Exp. Ther.*, 162, 294 (1968).
(21) N.-E. Anden, S. G. Butcher, H. Corrodi, K. Fuxe, and U. Ungerstedt, *Eur. J. Pharmacol.*, 11, 303 (1970).
(22) J. D. Dunitz, H. Eser, and P. Strickler, *Helv. Chim. Acta*, 47, 1897 (1964).
(23) J. R. Tretter, J. F. Muren, B. M. Bloom, and A. Weissman, Medicinal Chemistry Symposium of the American Chemical Society, Bloomington, Ind., June 1966.
(24) D. M. Gallant and M. P. Bishop, *Psychopharmacol., Rev. Progr.*, 1093 (1957–1967).
(25) J. P. Schaefer, *Chem. Commun.*, 743 (1963).
(26) C. Kaiser, R. J. Warren, and C. L. Zirkle, *J. Med. Chem.*, 17, 131 (1974).
(27) D. C. Remy and W. A. VanSaun, Jr., *Tetrahedron Lett.*, 27, 2463 (1971).
(28) F. Ullmann, *Justus Liebigs Ann. Chem.*, 355, 312 (1907).
(29) P. G. Sergeev, *J. Gen. Chem. USSR*, 1, 279 (1931); *Chem. Abstr.*, 26, 2184 (1932).
(30) P. N. Craig and C. L. Zirkle (to Smith Kline & French Labs.), U. S. Patent 3,192,204 (1965); British Patent 925,539; *Chem. Abstr.*, 59, 12766*d* (1963).
(31) P. N. Craig and C. L. Zirkle (to Smith Kline & French Labs.), U. S. Patent 3,282,930 (1966); *Chem. Abstr.*, 66, 46332a (1967).
(32) E. Bergmann and O. Blum-Bergmann, *Ber. Deut. Chem. Ges.*, 63, 757 (1930).
(33) S. O. Winthrop, M. A. Davis, F. Herr, J. Stewart, and R. Gaudry, *J. Med. Pharm. Chem.*, 5, 1207 (1962).
(34) K. Stach and H. Spingler, *Monatsh. Chem.*, 93, 889 (1962).
(35) G. Saucy and L. H. Sternbach, *Helv. Chim. Acta*, 45, 2226 (1962); *Chem. Abstr.*, 59, 1642*a* (1963).
(36) W. R. Waldron and E. E. Reid, *J. Amer. Chem. Soc.*, 45, 2399 (1923).
(37) M. Bogucka and Z. Ledochowski, *Rocz. Chem.*, 40, 677 (1966); *Chem. Abstr.*, 65, 5440 (1966).
(38) I. Tanasescu and E. Ramontianu, *Bull. Soc. Chim. Fr.*, 3, 2009 (1936).

Molecular Structure and Conformation of the Nucleoside Antibiotic Derivative 2-Methylformycin with a C-Glycosidic Bond

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The molecular and crystal structure of 2-methylformycin, a synthetic analog of the antibiotic formycin, has been solved using X-ray techniques. The space group is the monoclinic $P2_1$. Cell dimensions measured on the diffractometer are a = 9.208 Å, b = 14.367 Å, c = 4.791 Å, and β = 101.91°. The pertinent conformational values are as follows: the conformation about the C-glycosidic bond is syn, the torsion angle is −154.8°, the sugar pucker is C(3′) endo, a conformation usually observed only for nucleosides in the anti conformation, and the conformation about the C(4′)–C(5′) bond is gauche–gauche. Hydrogen bonding is observed between the base and ribose moieties. There is an intramolecular hydrogen bond between O(5′) and N(3) and no significant "base stacking" is observed in the molecular packing. Because of this preferred conformation in the solid state, it is speculated from biological testing data that 2-methylformycin is a poorer substrate of adenosine deaminase than formycin.

Structural elucidation studies established[1,2] that formycin, an antibiotic isolated[3] from *Norcardia interforma*, was a C-nucleoside isomeric with the naturally occurring purine nucleoside adenosine. Formycin has demonstrated[4] the ability to inhibit the growth of various experimental tumor cell lines, *Xanthomonas oryzae*, as well as exhibit some immunosuppressive activity.[5] Formycin has functioned[6,7] effectively in place of adenosine at the polymeric level and formycin has also functioned as a substrate for enzymes specific for adenosine kinase[8] and adenosine deaminase.[9] This close relationship to adenosine in substrate specificity and biological activity was of considerable interest since adenosine exists predominantly in the anti conformation while formycin hydrobromide was reported[2] to exist in the syn conformation. This conformation could have been attributed in part to protonation at N-8 (N-2) although the site of protonation was presumed[2] to be at N-1 (N-6)‡ (see Figure 1) on the basis of bond distance data. This is of interest in view of a recent study which has established[10] that adenosine derivatives in the syn conformation are not substrates for adenosine deaminase and would suggest that a significant population of formycin must exist in the anti conformation in solution and *in vivo*. In fact, it has now been established[11] that formycin (nonproponated) exists between the classical syn and anti conformations (amphi form[12]) in the solid state.

Structure analysis of 2-methylformycin by X-ray techniques was undertaken by us to obtain precise information on the structure and its conformation as a part of our current studies on the structures of nucleic acids, their components, and their cytotoxic analogs, as well as to gain some insight into the preferred conformations which might be related to the difference in biological testing and

†This paper is dedicated to my former professor, Alfred Burger.

‡We would like to point out that instead of following the numbering system normally used for this pyrazolopyrimidine system, we follow that used in purine systems (followed by the pyrazolopyrimidine numbering in parentheses). We elected to use this convention in order to facilitate a comparison of our observations with those of the native compound and other purine systems.

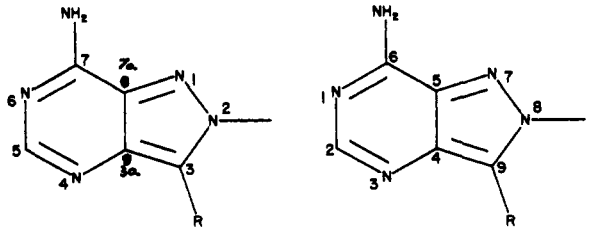

Figure 1. The numbering system used for the pyrazole pyrimidine ring is shown on the left, and the numbering system used in purines and followed in this paper is shown on the right.

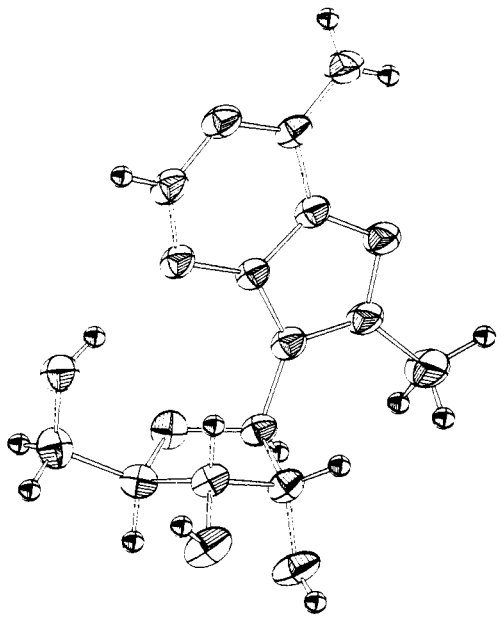

Figure 2. A computer plot of 2-methylformycin showing the thermal vibration ellipsoids of the heavy atoms.

binding to adenosine deaminase between formycin derivatives.

Experimental Section

Crystals of 2-methylformycin were grown in our laboratories. A needle-shaped crystal was mounted with the b axis parallel to the goniostat ϕ axis. The space group was determined by film methods to be the monoclinic $P2_1$. The cell dimensions measured in the diffractometer are a = 9.208 Å, b = 14.367 Å, c = 4.971 Å, and β = 101.91°. The crystal density was measured by floatation techniques and found to be 1.405 g/cc, agreeing well with a calculated density of 1.409 g/cc assuming two molecules per unit in the crystal.

Three-dimensional monochromated Cu Kα X-ray intensities were measured using the Nonius CAD-4 diffractometer using a θ-2θ scan. Reflections with their intensities less than three times their standard deviation were considered unobserved. The data were corrected for Lorentz and polarization effects but no corrections were made for absorption. Altogether 1381 independent reflections were scanned, of which 1354 were considered to be significantly above background.

Phases for reflections with E values greater than 1.3 where E is the normalized structure factor were generated using the multiple solutions program MULTAN.[13] Coordinates for all the nonhydrogen atoms were determined from a fourier map using E's with their calculated phases as coefficients. The positional as well as isotropic thermal parameters were refined using a full-matrix least-squares technique.[14] The Y coordinate of the N-1 (N-6) atom was held constant fixing the origin along the twofold screw axis. These atoms were then assigned anisotropic temperature factors and were subjected to one more cycle of refinement after which 13 hydrogen atoms were found in a difference fourier map. The coordinates of the hydrogen atoms and their isotropic temperature factors were then refined together with the anisotropic parameters of the nonhydrogen atoms. Convergence was assumed to have been achieved when the average ratio of the parametric shifts/estimated standard deviations for the nonhydrogen atoms was less than 0.3 and those for the hydrogen atom less than 1.0. The final R value was 0.029. Throughout the refinement each reflection was given a weight based on counting statistics. The scattering factors for carbon, nitrogen, and oxygen were those from Cromer and Waber,[15] while that of hydrogen was from Stewart, *et al.*[16]

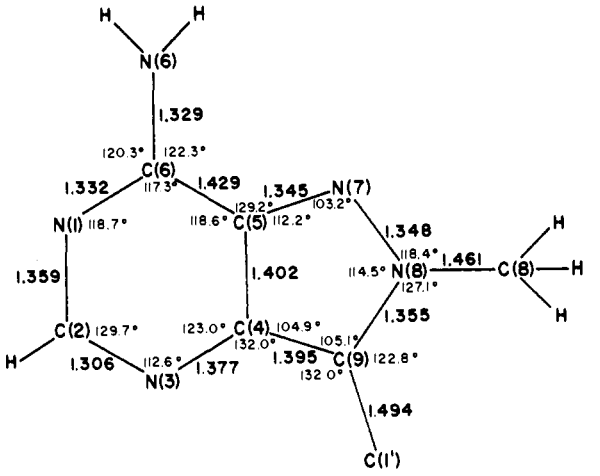

Figure 3. Bond lengths and angles in ångströms and degrees for the 2-methylpyrazolopyrimidine moiety of 2-methylformycin.

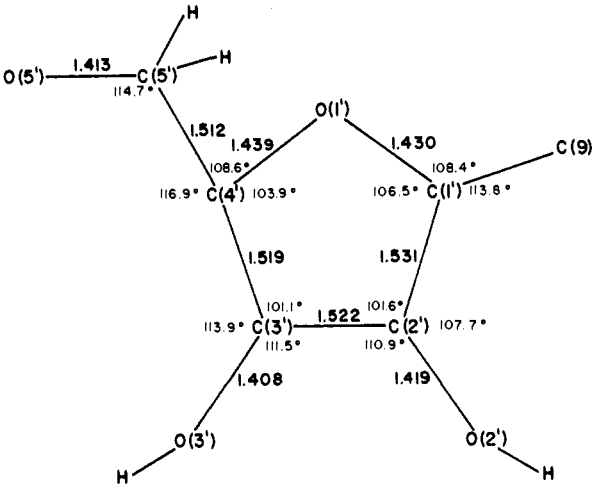

Figure 4. Bond lengths and angles in ångströms and degrees for the ribose moiety of 2-methylformycin.

Results and Discussion

The glycosidic torsion angle Xcc defined as the angle made by the projection of the C-8 (N-2)-C-9 (C-3) bond with respect to the C-1′-O-1′ bond is 154.8 (3)°. The conformation about the glycosidic bond can thus be described as being syn. The 2-methylation most probably locks the nucleoside into this conformation as there would be short interatomic contracts between the base and the sugar if the nucleoside were to assume the anti conformation.

The fractional coordinates and thermal parameters for both the hydrogen and nonhydrogen atoms were given in Table I (see paragraph at end of paper regarding supplementary material). A computer plot[17] of the molecule showing the thermal vibration ellipsoids of the atoms is shown in Figure 2. The hydrogen atoms in this figure were given an arbritrary isotropic temperature factor. A listing of the observed and calculated structure factor amplitudes will appear on the microfilm edition of this paper.

Figures 3 and 4 are schematic diagrams of the base and the ribose moieties showing the interatomic bond lengths and angles. The estimated standard deviation (esd) for all the bond lengths except that for the N-8 (N-2)-C-8 (C-2)

Table II. Hydrogen Bond Lengths and Angles in 2-Methylformycin[a]

Atoms			Symmetry code	A···B, Å	H···B, Å	H—A···B, deg
A	H	B				
N(6)	H'(N-6)	O(2')	2(1 − 10)	2.97	2.08	6.1
N(6)	H(N-6)	O(5')	2(0 − 1 − 1)	2.94	2.10	11.4
O(2')	H(O-2')	N(7)	2(100)	2.83	1.98	17.2
O(3')	H(O-3')	N(1)	2(00 − 1)	2.79	1.90	7.5
O(5')	H(O-5')	N(3)	1(000)	2.77	1.94	2.1

[a]Symmetry code: 1 (x, y, z); 2 $(-x, {}^1/_2 + y, -z)$.

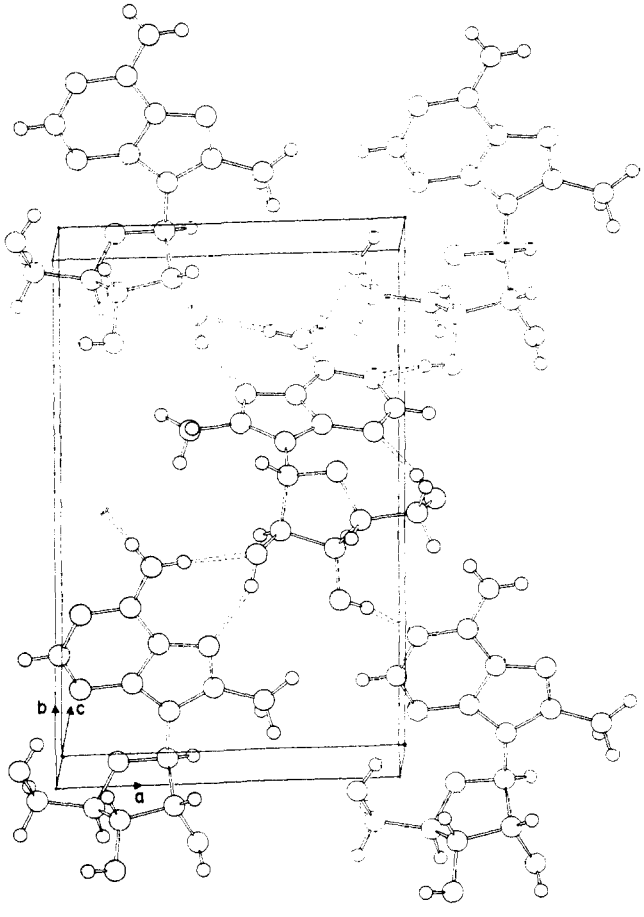

Figure 5. Hydrogen bonding scheme and molecular packing.

bond is 0.003 Å; the latter has an esd of 0.004 Å. The esd's for the bond angles are all 0.2°.

A comparison of the bond lengths and angles between the native compound,[11] formycin, and this derivative shows good agreement for the pyrimidine rings. Significant changes, however, are observed for the pyrazole ring. The most significant changes brought about by the N-methylation are a shortening of the bond across C-4 (C-3a)-C-9 (C-3) and a lengthening of the bonds across N-8 (N-2)-C-9 (C-3) and C-4 (C-3a)-C-5 (C-7a). This may be suggestive of a difference in the predominant resonance structures between the two molecules. Furthermore, comparing the bond lengths of the pyrimidine ring in both antibiotics with those observed in adenine systems,[18] we note a significant lengthening across the N-3 (N-4)-C-4 (C-3a) bond which seems to be a result of substituting a carbon for the nitrogen at the 9 position. Studies are currently being undertaken in our laboratory as to the significance of all these changes in bonding in the adenine moiety.

The sugar exhibits an envelope conformation with the C-3' atom displaced by 0.62 Å on the same side of C-5'. This puckering is also described as C-3' endo. Most sugars in which the nucleoside is in the syn conformation have been observed to exhibit the C-2' endo pucker,[19] and to our knowledge this is the first time that the C-3' endo pucker has been observed in syn nucleosides. The C-3' endo pucker, however, is that observed in nucleosides in the anti conformation.[19]

It is interesting to note that the conformation of the molecule as observed in this determination is consistent with the model assigned by Ward and Reich[20] to polyformycin (F). The optical rotary dispersion (ORD) studies on poly(F) revealed a curious "inverted spectra" for poly(F) relative to poly(A). This phenomenon was explained as possibly being due to the adoption of the classical syn conformation by the individual formycin residue in poly(F).

The conformation about the glycosidic bond as observed here is syn; furthermore, the conformation about the hydroxymethylene group on the ribose is gauche-gauche. The latter conformation is considered to be a property contributing to the helical nature of poly nucleotides.[21] It is possible, therefore, that the nucleotides on poly(F) assume the syn and gauche-gauche conformation, especially of the tautometric form in which N-8 (N-2) is protonated.

A recent cmr[22] study has established that formycin can exist in two predominant resonance forms with one of the resonance forms having a proton residing at N-8 (N-2) which would result in some restriction of rotation around the glycosyl bond as determined by CPK molecular models. Elimination of tautomerism in the pyrazole ring can be accomplished by replacing the proton with another group, *e.g.*, alkyl, and the introduction of a methyl group in formycin at N-8 (N-2) should provide a nucleoside predominantly in the syn conformation as determined by CPK molecular models. 2-Methylformycin§ has demonstrated‡ a higher level of activity against leukemia L-1210 than 1-methylformycin§ where the methyl group exerts no steric inhibition of rotation around the glycosyl bond. Therefore, on the basis of this preliminary antitumor evaluation, the formycin derivative in the syn conformation was more active than the formycin derivative with free rotation around the glycosyl bond, which would presumably assume a predominate conformation other than syn. This activity indicated that 2-methylformycin in the syn conformation (which we have now shown in the crystal state) was not deaminated or at least deaminated slower than 1-methylformycin and was a syn nucleoside which to some degree still functioned as a substrate for adenosine kinase although both of these assumptions must be corroborated by further study.

Hydrogen Bonding. The hydrogen bonding scheme of the structure is shown in Figure 5. The distances and angles are listed in Table II. Instead of reporting the internal hydrogen bond angle (A—H···B) as has been the practice, we are reporting the angle made around the hydrogen bond donor by the hydrogen atom and the hydrogen bond acceptor. Thus, angles near to 0° are linear. In this structure, we observe angles ranging from 2.1 to 17.2°. There

§A preliminary communication of the synthesis of 2-methylformycin has been reported; see ref 23.

‡Unpublished testing data from DR & D, DCI, NCI, to L. B. T.

are no hydrogen bonds between bases. All hydrogen bonds are between base and sugar. There is an intramolecular hydrogen bond between O-5′ and N-3 (N-4). This intramolecular interaction has been observed in purine nucleosides in the syn conformation, and it is thought to add to the stability of the molecule in its present conformation.[20] The exocyclic atom N-6 (N-7) donates its hydrogens to O-5′ and O-2′, whereas N-7 (N-1) accepts the hydrogen from O-2′ and N-1 (N-6) accepts a hydrogen from O-3′ is the only possible hydrogen bond acceptor that is not involved in the entire scheme. No significant "base stacking" is present in the molecule packing.

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Supplementary Material Available. Table I and a listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-74-62.

References

(1) R. K. Robins, L. B. Townsend, F. C. Cassidy, J. F. Gerster, A. F. Lewis, and R. L. Miller, *J. Heterocycl. Chem.*, **3**, 110 (1966).
(2) G. Koyama, K. Meada, J. Umezawa, and Y. Itake, *Tetrahedron Lett.*, 597 (1966).
(3) M. Hori, E. Ito, T. Takita, G. Koyama, and H. Umezawa, *J. Antibiot., Ser. A*, **17**, 96 (1964).
(4) R. J. Suhadolnik, "Nucleoside Antibiotics," Wiley-Interscience, New York, N. Y., 1970.
(5) T. Odaka, K. Takizawa, K. Yamaura, and T. Yamamoto, *Jap. J. Exp. Med.*, **39**, 327 (1969).
(6) D. C. Ward, A. Cerami, E. Reich, G. Acs, and L. Altewerger, *J. Biol. Chem.*, **244**, 3243 (1969).
(7) M. Ikehara, K. Murao, F. Harada, and S. Nishimura, *Biochem. Biophys. Acta*, **174**, 696 (1968).
(8) T. Sawa, Y. Fukagawa, U. Shimauchi, K. Ito, M. Hamada, T. Takeuchi, and H. Umezawa, *J. Antibiot., Ser. A*, **18**, 259 (1965).
(9) T. Sawa, Y. Fukagawa, I. Homma, T. Takeuchi, and H. Umezawa, *ibid.*, **20**, 317 (1967).
(10) K. K. Ogilve, L. Slotin, and P. Rheault, *Biochem. Biophys. Res. Commun.*, **45**, 297 (1971).
(11) M. Sundaralingham, *Biochemistry*, in press.
(12) M-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Heterocycl. Chem.*, **10**, 427 (1973).
(13) P. Main, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).
(14) W. R. Busing, K. A. Martin, and H. A. Levy, Oak Ridge National Laboratory Report ORNL-TM-305, Oak Ridge, Tenn., 1962.
(15) D. T. Cromer and J. T. Waber, *Acta Chrystallogr.*, **18**, 104 (1965).
(16) R. F. Steward, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(17) C. K. Johnson, Oak Ridge National Laboratory Report ORNL-3794, Oak Ridge, Tenn., 1965.
(18) S. T. Rao and M. Sundaralingam, *J. Amer. Chem. Soc.*, **92**, 4963 (1970).
(19) M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).
(20) D. C. Ward and T. Reich, *Proc. Nat. Acad. Sci. U. S.*, **61**, 1494 (1968).
(21) M. Sundaralingam, *Symp. Quant. Chem. Biochem., 5th*, 417 (1973).
(22) M-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Heterocycl. Chem.*, **10**, 431 (1973).
(23) R. A. Long, L. B. Townsend, D. W. Miles, H. Eyring, and R. K. Robins, Abstracts, 161st National Meeting of the American Chemical Society, Los Angeles, Calif., March 25, 1971.

Antidepressant Agents. 1. Chemistry and Pharmacology of Amino-Substituted Spiro[5*H*-dibenzo[*a*,*d*]cycloheptene-5,1′-cycloalkanes]

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A series of spiro compounds structurally related to the common tricyclic antidepressants has been tested as inhibitors of the neuronal reuptake of noradrenaline and 5-hydroxytryptamine. Also, some behavioral tests have been performed. Two of the substances, *N,N*-dimethylspiro[5*H*-dibenzo[*a*,*d*]cycloheptene-5,1′-cyclohex-2′-en]-4′-amine (**11**) and *N,N*-dimethylspiro[5*H*-dibenzo[*a*,*d*]cycloheptene-5,1′-cyclohexan]-4′-amine (**18**), are very active in the NA-uptake inhibition assay. A discussion on structure–activity relationships is given.

Tricyclic antidepressant agents show a variety of pharmacological properties, *i.e.*, inhibition of the presynaptic uptake of noradrenaline (NA) and 5-hydroxytryptamine (5-HT), prevention of the reserpine syndrome, anticholinergic effect, and cardiotoxic effect.[1] This lack of specificity may be explained by the multitude of possible conformations of these drug molecules. The tricyclic antidepressants consist of a condensed three-ring system connected to a three-carbon side chain which is terminated by a tertiary or a secondary amino group.[2] The side chain has a considerable degree of freedom due to rotation around the single bonds and the terminal amino group can therefore occupy a great number of positions in relation to the tricyclic skeleton.

In our search for selective antidepressants it was consequently considered to be of interest to study compounds where the terminal amino group is locked in a defined position. This was achieved by letting the side chain participate in a ring system of rigid structure.

We have now synthesized a series of amino-substituted spiro[5*H*-dibenzo[*a*,*d*]cyclohexanes] and spiro[5*H*-dibenzo[*a*,*d*]cyclopentanes] in which the structural features of

†This paper is dedicated to my former professor, Alfred Burger.