jection of carrageenin into the hind paw of the rat was employed to measure the antiinflammatory potency of these substances.[5] A citric acid spray in the guinea pig was used to test the possible antitussive activity.[6]

Tables I and II state the results of biological studies. Substances of the benzamides series were only tested as potential antiinflammatory compounds, with phenylbutazone as a standard. Substances of the (1*H*)-quinazolinones series were tested as analgetic and antiinflammatory agents. Codeine phosphate was employed for comparison in the hot plate test. Among the compounds studied, none was equal to phenylbutazone. Only compounds **20**, **22**, **34**, and **38** were equal to or better than codeine phosphate as analgetic agents.

The most interesting compound was **20**, which is twice as active as codeine phosphate after parenteral or oral administration in analgetic and antitussive screening assays. The LD_{50} for this substance administered intragastrically to mice is 438 mg/kg (400–476).[7] Neither respiratory failure nor ataractic effect were observed in laboratory animals after the administration of effective analgetic doses of the compound, which was selected for preliminary clinical trials.

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Experimental Section

2-(Alkenylamino)benzamides (IV). Table I. Method A.—Adapted from ref 2. To a stirred soln of the amide II (2 moles) in DMF contg anhyd Na_2CO_3 (2 moles) and maintained at 30° by external cooling, was added the alkenyl bromide (2 moles) over a period of 0.75 hr. Stirring was contd for 24 hr, the mixt was poured into cracked ice (2.5 kg) and the ppt formed was filtered, washed (H_2O) to neutrality, air-dried, and crystd from the appropriate solvent.

Method B.—Adapted from ref 3. To stirred, concd NH_4OH (2 moles of NH_3 in 135 ml) was added portionwise the isatoic anhydride III (0.25 mole) over a period of 0.25 hr. The thick suspension was dild with H_2O (50–100 ml) so that it could be stirred easily and stirring was contd for 15 hr. The ppt was suctioned off, washed (H_2O) to neutrality, dried under vacuum (10 mm) at 50°, and crystd from an appropriate solvent.

1-(Alkenyl)-4-(1*H*)-quinazolinones (V). Table II. Method C.—Adapted from ref 1. A soln of the amide IV (2 moles) in ethyl *o*-formate (2 l) was kept boiling for 35 hr while distg off the EtOH formed. The mixt was then stirred for 15 hr at room temp. The ppt formed was suctioned off, washed with ethyl *o*-formate (20–50 ml), then with pentane (100–200 ml), dried, and cryst. When the product did not cryst from ethyl *o*-formate, the excess of solvent was distd off (water bath, 10 mm), and the residue was crystd from an appropriate solvent.

***cis*-2-(2-Cyclohexenylamino)benzamide (6).**—Prepd by method A, the product crystd from DMF–H_2O as a solvate contg 1 mole of DMF per mole of amide. It also gave a solvate with C_6H_6 and had to be purified by pptg the HCl salt from Et_2O, decompg the salt with the calcd amt of NaOH in H_2O, filtration, and drying.

Conformational Studies of Amphetamine and Medicinally Important Derivatives by Nuclear Magnetic Resonance Spectroscopy†

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Conformational analysis of amphetamine free base (I), amphetamine·HCl (II), methamphetamine·HCl (III), *o*-methoxymethamphetamine·HCl (IV), and benzphetamine·HCl (V) by high-resolution nmr spectroscopy has demonstrated a high preference for the *trans*-phenylamino rotamers (Ia, IIIa, IVa, Va) in aq solution. Since the same trans conformational preference was recently established for norepinephrine (NE), the prototype for α-adrenergic catecholamines, this structural evidence is compatible with the increasingly popular view that amphetamines may exert their pharmacological activity as α-adrenergic agonists. Evidence has been obtained for the first time that intramolecular H bonding occurs in methoxyphenamine·HCl (IV) between an ammonium proton and the O of the *o*-OMe substituent. Amphetamine·HCl (II) was found to give a "deceptively simple" ABC spectrum in H_2O.

In recent years, various ring-substituted amphetamine derivatives have been studied in humans by Shulgin[1] and in animals by Smythies, *et al.*,[2,3] to derive structure–activity relationships. From human dose–response relationships for psychotomimetic phenethylamines, Shulgin, *et al.*,[4] have concluded that optimum activity is conferred by an isopropylamine side chain and triple MeO substitution. A correlation between hallucinogenic activity of drugs and their electronic

† Issued as NRCC No. 11971.
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configuration was developed by Snyder and Merril[5] which predicted relative activity in given classes of drugs but could not take into account differences in pharmacological activity among the different structural classes of hallucinogenic drugs. More recently, Snyder and Richelson[6] proposed a common molecular configuration to account for the similar action of different classes of some hallucinogens on the basis of assumed mimicry of either ring B or C of the LSD molecule. Chothia and Pauling[7] dismissed the latter correlation as unreasonable and proposed instead that the most probable conformation for 3 classes of hallucinogenic molecules would be that deduced by assuming normal stereochemical rules.

All conformational proposals made to date for substituted amphetamines have been speculated rather than determined experimentally. The prediction by Chothia and Pauling that the isopropylamine side chain of 2,4,5-trimethoxyamphetamine is antiplanar and perpendicular to the plane of the ring[7] is supported by evidence from solid-state studies indicating the same configuration in crystals of phenylethylamine[8] and dopamine·HCl.[9] While the conformation imposed by the crystal lattice may also be the preferred conformation in the solvated state, a population distribution of all possible rotamers is more frequently encountered in solution. Nmr spectroscopy is often ideally suited for providing information concerning the molecular geometry of solvated molecules by analysis of vicinal proton coupling and application of the Karplus relationship.[10] Very few compounds structurally similar to amphetamine and of medicinal interest have received conformational study by nmr spectroscopy, mainly due to the complexity of the spectra. Some relevant examples of the procedure include an analysis of chloramphenicol,[11] determination of the preferred conformations of the related diastereoisomeric pair, ephedrine and pseudoephedrine,[12,13] and analysis of the rotamer populations of (−)-*N*,*N*-dimethyl-1,2-diphenylethylamine[14] and of the trimethylsilyl ethers of some β-hydroxyphenylalkylamines.[15]

Experimental Section

Nmr spectra of amphetamine (α-methylphenethylamine) (I), amphetamine·HCl (II), methamphetamine·HCl (*N*-methylamphetamine·HCl) (III), methoxyphenamine·HCl (*o*-methoxymethamphetamine·HCl) (IV), and benzphetamine·HCl (*N*-benzyl-*N*,α-dimethylphenethylamine·HCl) (V) were obtained in D_2O at 100 MHz on a Varian XL-100 spectrometer at an ambient temperature of 30°. The amphetamine (free base) was freshly distd [bp 44° (0.3 mm)] whereas the remaining compds, being of pharmaceutical quality, were used as received. All samples with the exception of I were examined in D_2O at concns of approximately 100 mg of solute/ml of solvent. Amphetamine free base (I) was examined as a saturated aq soln. Chemical shifts and coupling constants are reported in Table I. No concn dependence was observed on diln of the samples. Spectral analyses were performed with the aid of a modified version of the computer program LAOCOON II.[16,17]

Results and Discussion

Analysis of the Spectra.—The spectral features arising from the CH_2 and CH protons of I–V constitute ABC systems[18] but may be approximated in the preliminary analysis as ABX systems. In the final analysis, the systems were treated as ABC to obtain more accurate parameters. In all instances, the X or C portion of the pattern due to the CH proton was found downfield of the AB portion (CH_2) and was observed as a complicated multiplet from vicinal coupling of the CH proton with the α-Me protons.

In considering the analysis of the 8-line AB portion (Figure 1), there are 2 ways of choosing the 2 pseudo AB quartets[19,20] each choice leading to two solutions depending on whether wide or narrow coupling[20] is invoked. Since wide coupling and the case of eclipsed narrow coupling each require that the signs of J_{AX} and J_{BX} be unlike, the AB portion was analyzed as a partially eclipsed system with narrow couplings which leads to like signs for J_{AX} and J_{BX}. In the analysis J_{AX} and J_{BX} were assumed to have like signs and to be positive since it has been shown that the vicinal coupling constants in substituted ethanes have the same sign[21–23] and are regarded as positive.[24] In addition, J_{AB} was assumed to be negative since for most CH_2 groups, the geminal proton coupling is negative.[25] The validity of the above choice of signs was verified by computer simulation of the X portion of the spectra. These assumptions are also in accord with the known molecular structure where it is reasonable to rule out the likelihood of two large couplings of opposite signs for J_{AX} and J_{BX} by analogy with related systems.[12–14,26] A typical nmr spectrum and that simulated using the parameters obtained by computer analysis is presented in Figure 1.

Rotational Isomerism and Population Distribution.—From the coupling between the CH and CH_2 protons, it is possible to estimate the relative populations of rotational isomers. These vicinal proton coupling constants are averaged values and should be designated as $\bar{J}_{AC}$ and $\bar{J}_{BC}$. These averaged values may be considered to arise from a population distribution among "60° staggered" rotamers like Ia, Ib, and Ic, illustrated for amphetamine (Figure 2), in which the C proton bears either a trans or gauche relationship to the A and

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TABLE I

CHEMICAL SHIFTS[a] (PPM) AND COUPLING CONSTANTS (HZ) OBTAINED BY ABC ANALYSES OF SPECTRA AT 100 MHZ

Compd	H_A	H_B	H_C	α-CH_3	J_{AB}	J_{AC}	J_{BC}	$J_{CH_3(HC)}$
Amphetamine (I)[b]	2.58	2.70	3.12	1.05	−13.30	7.50	6.28	6.40
Amphetamine·HCl (II)[c]	3.26[d]	3.26[d]	3.95[e]	1.61[e]	−13.8[d]	7.4[d]	6.9[d]	6.60[e]
Methamphetamine·HCl (III)[f]	2.90	3.10	3.54	1.28	−13.72	8.08	6.25	6.50
Methoxyphenamine·HCl (IV)[f]	2.92	3.05	3.55	1.25	−13.75	7.19	6.00	6.40
Benzphetamine·HCl (V)[c]	3.24	3.51	4.03	1.63	−13.46	9.07	5.80	6.60

[a] At 32° the principal peaks of the internal references DSS and TSP are 0.36 and 0.33 ppm downfield from external HMDS, respectively, as measured on an A60 nmr spectrometer. [b] Measured relative to internal 0.5% DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate). [c] Measured relative to HMDS (hexamethyldisiloxane) in a coaxial capillary tube. [d] Obtained by extrapolation of data from DMSO-d_6–D_2O mixtures (see text). [e] Obtained directly from spectra in D_2O. [f] Measured relative to internal 0.5% TSP (sodium 3-trimethylsilylpropionate-*2,2-3,3-d*$_4$).

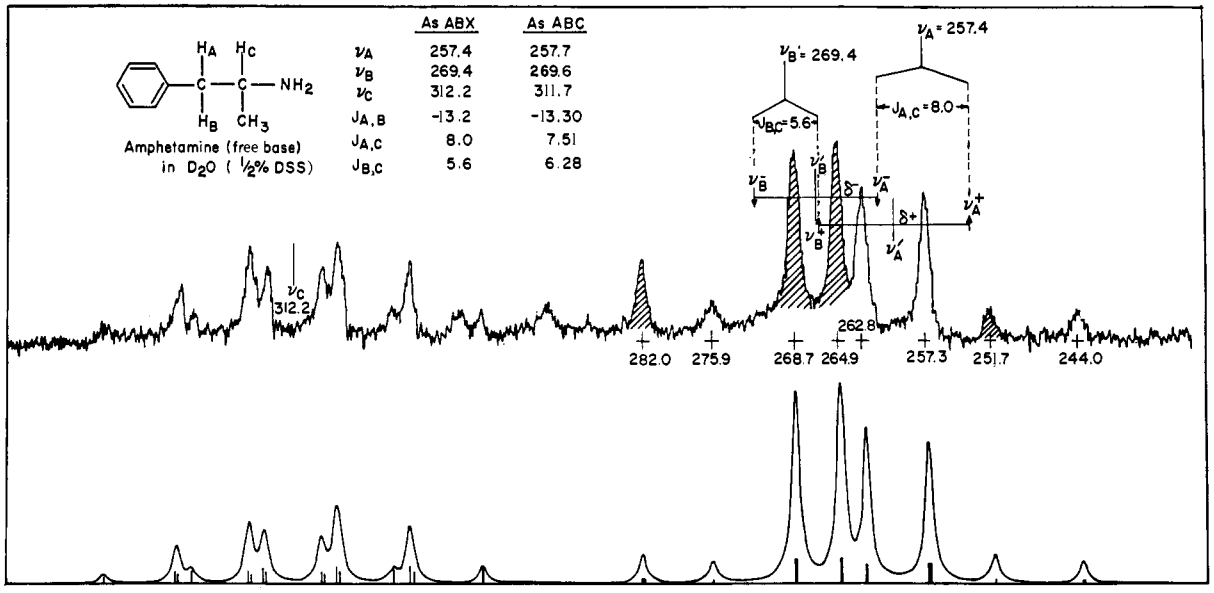

Figure 1.—The ABC portion of the nmr spectrum of amphetamine (free base) in D_2O obtained at 100 MHz showing preliminary analysis as an ABX system, followed by computer analysis and simulation as an ABC system.

B protons. Alternatively, the averaged values $\bar{J}_{AC}$ and $\bar{J}_{BC}$ may be considered to arise from a distribution of population among "off-staggered" rotamers[12,13] for as Hyne[12] has observed, there is no compelling reason to assume that the classical "pure staggered" conformations represent energy minima on the energy *vs.* angle of a rotation diagram. In this case, it seems that the 60° conformer shown in Newman projection in Figure 2 will be a very good approximation to the energy minima because the various repulsive and attractive forces act in opposite senses across the ethane axis and will, therefore, tend to be annulled.

Expressions for the average values $\bar{J}_{AC}$ and $\bar{J}_{BC}$ may be written in terms of J_{trans} (J_t) and J_{gauche} (J_g) and population-weighting factors (P) by reference to Figure 2; thus we obtain $\bar{J}_{AC} = P_aJ_t + P_bJ_g + P_cJ_g$ and $\bar{J}_{BC} = P_aJ_g + P_bJ_t + P_cJ_g$.

Solving each of these expressions with the population function, $P_a + P_b + P_c = 1$, gives the following equations for rotamer population $P_a = (\bar{J}_{AC} - J_g)/(J_t - J_g)$ and $P_b = (\bar{J}_{BC} - J_g)/(J_t - J_g)$. In order to evaluate the rotamer population factors, average values of J_g = 2.0 and J_t = 13.0 Hz were taken from a recent graph[27] of the "Karplus relation" constructed from a set of extreme values for J^0 and J^{180} for the case of not more than one strongly electronegative substituent. The calcd population distribution for rotamers a, b, and c of each of the compounds I–V in D_2O at 30° is summarized in Figure 2. Calculations were also performed allowing J_g values of 1.5 and 2.5 Hz for the two possible gauche rotamers, and J_t values of 12.5 and 14.0 Hz. The preference for the *trans*-phenylamino rotamer (Ia) was predicted in each case.

Discussion of Conformational Preference.—Conventional stereochemical considerations led to the prediction that amphetamine free base would have a preference for the *trans*-phenylamino rotamer (Ia). This prediction is verified by the data of Figure 2. The validity of the approach by which this conclusion was reached is substantiated by the trends noted for the N-substituted analogs (III–V), where increasing bulkiness of the N-substituent results in increasing preference for the trans rotamers (IIIa, IVa, Va). H_2O is expected to act as a proton donor to the lone pair of the amino moiety in solvating amphetamine free base. In the case of amphetamine salts where the amino N is protonated and acts as a proton donor, H_2O is expected to reverse its H bonding role by utilizing its O atom as a proton acceptor. The H bonding of H_2O in this fashion to a proton of the ammonium N in protonated amphetamines would consequently lead to greater branching in the molecular structure of solvating H_2O than is possible with amphetamine free base. On this basis one might expect amphetamine·HCl in D_2O to show evidence for a higher conformational preference for the

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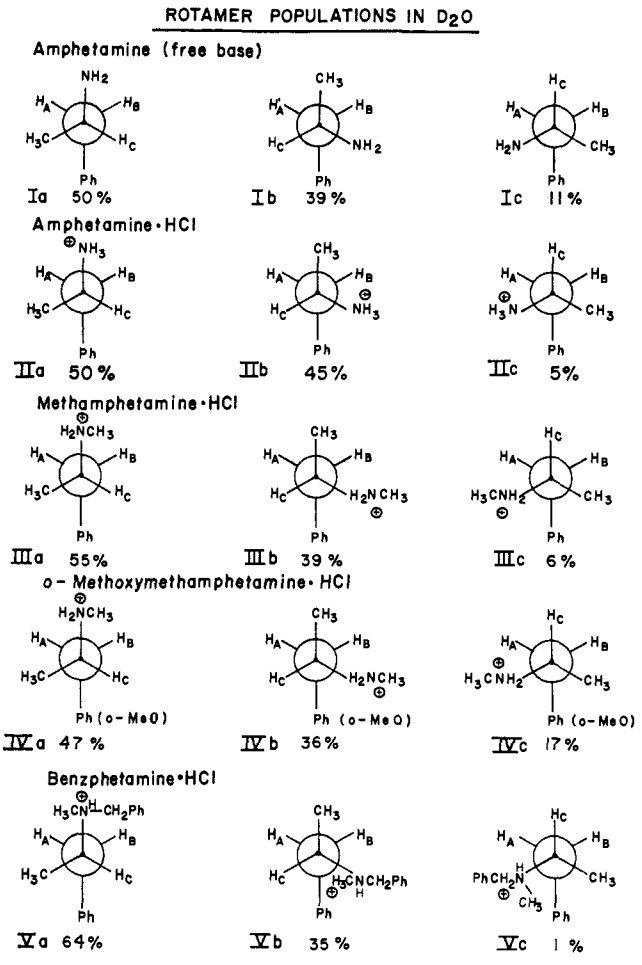

Figure 2.—Rotamer populations deduced from values of J_{AC} and J_{BC} (Table I), J_g = 2.0 and J_t = 13.0 Hz for "60° conformers."

trans-phenylammonium rotamer IIa than the free base. Such information is not easily obtained, however, for spectra of amphetamine·HCl in D_2O at different field strengths were all found to be "deceptively simple"[20] with only 2 lines being observed in the AB region. It is not possible, therefore, to obtain $\bar{J}_{AC}$ and $\bar{J}_{BC}$ singly[20,28] as required for calculation of rotamer distribution. The fact that such degeneracy arises when the difference in chemical shifts for the A and B protons approaches zero suggests that the A and B protons of the most heavily populated rotamer are nearly isochronous. Since none of the rotamers present a nearly similar chemical environment for each of the A and B protons, the deceptively simple spectral character may reflect a more evenly balanced rotamer distribution for amphetamine·HCl. This conclusion was verified by studying II in mixtures of DMSO-d_6 and D_2O of varying proportions. Increasing DMSO-d_6 concn resulted in a decreased preference for the IIb and IIc rotamers. Extrapolation of the coupling constants and chemical shifts of these easily analyzed spectra to solution in D_2O alone resulted in the parameters given in Table I. Spectral simulation confirmed the analysis.

In the case of protonated N-substituted amphetamine derivatives, the dominant steric effect appears to arise from N-substitution rather than from H_2O of solvation. The rotamer distribution of methamphetamine·HCl (III) in D_2O is insignificantly different from that for amphetamine free base (I) in D_2O even though the former compound bears an *N*-Me substituent. The addition of the larger *N*-benzyl substituent as in benzphetamine·HCl (V) results in a still higher preference for the trans rotamer Va at the expense of the population of the gauche rotamer Vb. The presence of an *o*-MeO substituent in methoxyphenamine·HCl (IV) results in a small but significant increase in the population of the gauche rotamer IVc in D_2O at the expense of the population of the trans rotamer IVa. Since III and IV have identical N-substitution, the small, relative increase in gauche rotamer population of IVc is evidence for the existence of intramolecular H bonding of an ammonium proton with the O atom of the MeO substituent as proposed earlier by Snyder and Richelson.[6] The predominant conformation of methoxyphenamine·HCl (IV) in D_2O, like that for all other amphetamine salts examined here, is the *trans*-phenylammonium rotamer IVa.

Relevance of Conformational Preference to Pharmacological Activity.—Amines such as tyramine, phenethylamine, amphetamine, and *N*-methylamphetamine have been classified as "indirectly acting" sympathomimetic amines following the observations by many workers that preadministration of reserpine to animals results in decreased activity of these amines.[29] Amphetamine and its *N*-alkyl derivatives exert marked excitatory effects on the CNS. There have been many theories to explain this action including synaptic release of the adrenergic mediator, norepinephrine (NE),[30] inhibition of the reuptake of NE,[31] inhibition of monoamine oxidase (MAO),[32] and direct action on receptors of the brain.[33] Although the indirectly acting amines release small amounts of NE into the circulation, it is questionable whether these quantities fully account for the powerful sympathomimetic effects observed.[29] More recently, new evidence has been presented suggesting that inhibition of NE uptake may be a major mechanism of action[34] whereas methamphetamine has been shown to interfere with the deamination of NE by MAO.[35]

There appears to be an increasing recognition of the possible direct action of amphetamine at brain receptor sites regarded as α-adrenergic in nature.[36] The ability of amphetamine to produce central stimulation even after NE depletion by reserpine may be related to such a direct effect of amphetamine on postsynaptic sites.[30c,33] In studying the uptake of normethanephrine by brain slices as a model for the accumulation of NE at or near postsynaptic sites, Hendley and Snyder[37]

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observed that psychotropic methoxyamphetamines were potent inhibitors of this uptake process and that their ability to inhibit normetanephrine transport correlated closely with their potency as hallucinogenic agents. From this evidence, Hendley and Snyder suggested that hallucinogenic drugs act at noradrenergic receptors of the brain.

In terms of drug–receptor complexation, any one drug conformation would be stabilized relative to the other on interaction with the receptor site. Generally, it is assumed that noncovalent interaction occurs in the form of the preferred conformation. MO calculations have been performed by Kier to deduce the preferred conformation of 5-hydroxytryptamine, whose calculated inter-N distance was found to correspond to the inter-N distance in the potent antagonist LSD,[38] and of norepinephrine[39] whose preferred conformation was found to be identical with the previously calculated conformation of ephedrine[40] in respect to the relation of the quaternary N and OH groups and the Ph ring. Since these calculations have resulted in the same preferred conformation as deduced by other workers from either crystal or solution data, the consistency of evidence tends to support the view that these molecules function at the receptor site in their preferred conformation.

Norepinephrine and epinephrine, being prototypes for the α-adrenergic catecholamines, have been used as models to postulate the essential features of α-adrenergic receptor sites.[39-41] In view of the fact that amphetamine free base and the protonated amphetamine derivatives all show the same conformational preference for the *trans*-phenylamino rotamer (*e.g.*, Ia) in D_2O as that determined by Kier for NE, the conformational evidence, at least, is compatible with the view that amphetamines may exert their pharmacological activity as α-adrenergic agonists. It is also apparent from the trend of these conformational studies that various ring-substituted amphetamine derivatives should also show conformational preference for the *trans*-phenylamino rotamer. The relative hallucinogenic activity of these substituted amphetamines, having approximately the same drug–receptor geometry in which a low energy, reversible π-molecular complex presumably forms with brain receptor, may well depend on the suggested ease of perturbability of the π electrons of the Ph ring.[5,42]

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A New Series of Semisynthetic Rifamycins. N Derivatives of 4-Amino-4-deoxyrifamycin SV†

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The preparation and the chemical and physical properties of a new series of semisynthetic rifamycins are described. These N derivatives of 4-amino-4-deoxyrifamycin SV are obtained by reaction of rifamycin O with secondary amines. Spectral evidence is presented for the structures of the title compounds. The antibacterial activity of the new compounds is reported and structure–activity correlation is briefly discussed.

The substitution of various functional groups in positions 3 and/or 4 of rifamycin SV (Ia) has yielded many active derivatives.[1] Among them, rifampicin‡[2] has been successfully introduced into clinical use for the treatment of the tuberculous Gram-positive and Gram-negative infections.

The possibility of obtaining a new series of rifamycins (Ib), substituted in position 4 with the group NR_2, has now been explored, by allowing rifamycin O (II) to react with primary, secondary, and tertiary amines in the aliphatic, aliphatic–aromatic, or cyclic series.

Initial attempts revealed that the reaction with primary amines produced a very complicated mixture of products which could not be completely separated and purified by column chromatography. The pmr spectra of some of these products showed that rifamycin was extensively degradated. The reaction with tertiary amines gave unchanged starting material; therefore the reaction with secondary amines has been studied.

This reaction is performed by treating rifamycin O with an excess (2:1) of the amine in THF at room temp. After a period of 12 to 120 hr according to the reaction rate, tlc (silica gel HF_{254} Merck, $CHCl_3$–MeOH, 9:1) of the reaction mixture shows the disappearance of rifamycin O and the formation of a new product, with lower R_f than that of rifamycin O, together with a moderate quantity of rifamycin B and trace amounts of by-products. The formation of rifamycin B is due to the reducing power of amines; this is not surprising because rifamycin O is easily reduced to rifamycin B by reducing agents.[3] At a higher temp, there was no improvement in yields, because degradation of rifamycin gave a series of by-products.

Only the product derived from the reaction of rifamycin O with Me_2NH (**1**, Table I) could be obtained directly by crystallization from EtOAc. In all other cases (**2–15**, Table I), the new products were obtained amorphously by precipitation with hexane from EtOAc. The yields were of the order of 20%. Physicochemical

† Rifamycins 64.

‡ Rifampicin is the common name (by the World Health Organization) for 3-(4-methylpiperazinyliminomethyl)rifamycin SV. The name adopted by USAN for the same compound is rifampin.

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