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]

(38) L. B. Kier, *J. Pharm. Sci.*, **57**, 1188 (1968).
(39) L. B. Kier, *J. Pharm. Pharmacol.*, **21**, 93 (1969).
(40) L. B. Kier, *J. Pharmacol. Exp. Ther.*, **164**, 75 (1968).
(41) B. Belleau, *Pharmacol. Rev.*, **18**, 131 (1966).
(42) S. Kang and J. P. Green, *Nature (London)*, **226**, 645 (1970).

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

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Received February 17, 1971

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

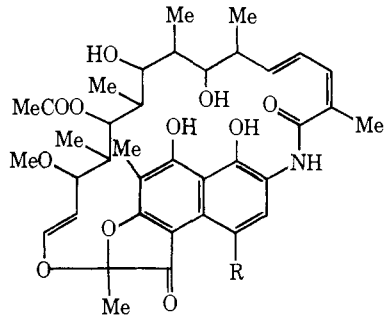
† Rifamycins 64.

‡ 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.

(1) P. Sensi, N. Maggi, S. Furesz, and G. Maffii, *Antimicrob. Ag. Chemother.*, 699 (1966).
(2) N. Maggi, C. R. Pasqualucci, R. Ballotta, and P. Sensi, *Chemotherapia*, **11**, 285 (1966).
(3) P. Sensi, R. Ballotta, and A. M. Greco, *Farmaco Ed. Sci.*, **15**, 228 (1960).

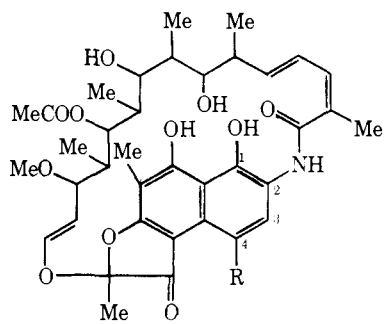
TABLE I

N DERIVATIVES OF 4-AMINO-4-DESOXYRIFAMYCIN SV

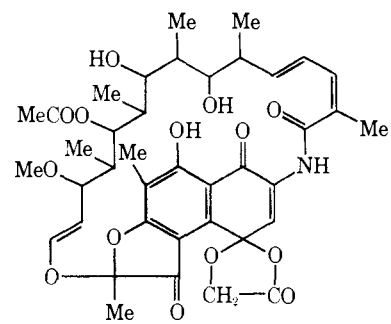


Compd	R	Yield, %	Mp, °C[a]	Formula[c]	Spectrophotometric data[d]			
					λ_{max}, nm	ϵ	λ_{max}, nm	ϵ
1	NMe_2	20	167–178	$C_{39}H_{52}N_2O_{11}$	302	18.800	418	22.000
2	NEt_2	20	176–178	$C_{41}H_{56}N_2O_{11}$	305	14.800	424	10.900
3	N-*n*-Pr_2	13.5	150[b]	$C_{43}H_{60}N_2O_{11}$	303	16.800	420	19.800
4	N-*n*-Bu_2	20	133–136	$C_{45}H_{64}N_2O_{11}$	302	17.100	420	19.800
5	$N(CH_2CH{=}CH_2)_2$	8.4	145–150	$C_{43}H_{56}N_2O_{11}$	303	16.800	418	20.000
6	$N(CH_3)CH_2C_6H_5$	25	139–155[b]	$C_{45}H_{56}N_2O_{11}$	305	16.600	420	18.400
7	$N(C_2H_5)CH_2C_6H_5$	25	155–180[b]	$C_{46}H_{58}N_2O_{11}$	300	17.700	416	19.800
8		15	165–175	$C_{42}H_{56}N_2O_{11}$	302	18.600	415	20.500
9		13.5	173–180	$C_{43}H_{58}N_2O_{11}$	303	18.300	420	19.400
10		13.5	178–200[b]	$C_{43}H_{58}N_2O_{11}$	304	23.600	424	19.500
11		13.5	165–174	$C_{43}H_{58}N_2O_{11}$	302	20.200	416	19.700
12		20	170–176	$C_{41}H_{54}N_2O_{12}$	303	17.200	420	19.400
13		13.5	185–190	$C_{41}H_{53}N_3O_{11}$	303	17.000	416	15.400
14		10	162–164	$C_{44}H_{59}N_3O_{13}$	303	17.500	420	18.600
15		30	167–171	$C_{42}H_{57}N_3O_{11}$	302	17.800	420	19.000

[a] All melting points were determined in open glass capillaries, using a Büchi apparatus, and are uncor. [b] Decd. [c] All compds were analyzed for C, H, and N. The anal. results were within ±0.4% of the theoretical values. [d] In phosphate buffer, pH 7.38.



Ia, R = OH, rifamycin SV
Ib, R = NR′R″, *N* derivatives



II, rifamycin O

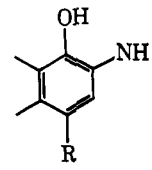
characteristics of the compounds are reported in Table I. These rifamycins are yellow and moderately sol both in acidic (0.1 *N* HCl) and in basic medium (satd soln of $NaHCO_3$); very sol in common organic solvents.

Their structure has been established through physical data, (pmr, mass, ir, uv, and visible spectra and polarographic analysis). The pmr spectrum of these compounds was interpreted on the basis of the assignments made by Prelog, *et al.*, in the course of the structure elucidation of rifamycin S.[4] The main features are the disappearance of the signals of the CH_2 group of the spirolactone ring of the rifamycin O at δ 4.64, the appearance of the new signals due to the dialkylamino group; and the shift of the singlet of the aromatic H to lower fields (δ 8.82). The mass spectrum, taken

(4) W. Oppolzer, V. Prelog, and P. Sensi, *Experientia*, **20**, 335 (1964).

TABLE II

ANTIBACTERIAL ACTIVITY OF N DERIVATIVES OF 4-AMINO-4-DESOXYRIFAMYCIN SV



	Minimal inhibitory concentration, μg/ml							
Compd	*Staphylococcus aureus* 209 P	*Streptococcus hemolyticus* C 203	*Diplococcus pneumoniae* UC 41	*Proteus vulgaris* ATCC 881	*Escherichia coli* ATCC 10536	*Klebsiella pneumoniae* ATCC 10031	*Pseudomonas aeruginosa* ATCC 10045	*Mycobacterium tuberculosis* H 37 Rv ATCC 9360
1	0.005	0.005	0.01	5	5	5	5	0.05
2	0.01	0.02	0.02	50	20	20	20	0.05
3	0.05	0.1	0.1	50	>50	>50	>50	0.2
4	0.1	0.02	0.05	>100	>100	>100	>100	0.2
5	0.02	0.02	0.02	20	50	10	20	0.1
6	0.02	0.01	0.01	10	20	20	20	0.1
7	0.05	0.02	0.02	>50	>50	>50	>50	0.1
8	0.005	0.05	0.05	50	10	10	10	0.1
9	0.02	0.05	0.05	>50	20	20	20	0.05
10	0.02	0.01	0.005	100	>100	>100	>100	0.2
11	0.01	0.02	0.02	50	10	10	10	0.1
12	0.01	0.002	0.002	10	10	10	10	0.1
13	0.01	0.02	0.005	5	2	5	10	0.1
14	0.02	0.005	0.01	50	20	20	20	0.1
15	0.02	0.01	0.01	10	10	5	10	0.1
16[a]	0.005	0.002	0.002	25	50	25	50	0.05

[a] Rifamycin SV, for comparison (R = OH).

with the NMe_2 derivatives only, shows *m/e* 724, in agreement with the calcd molecular weight.

The ir spectrum shows the disappearance of the absorption band at 1822 cm^{-1}, which is characteristic of the spirolactone ring of rifamycin O.[5] The polarographic analysis does not show the reduction wave ($E_{1/2}$ = +0.03 V *vs.* sce) of the system 1-4 quinone ⇄ hydroquinone, characteristic of rifamycin of type SV.[6] Moreover the chromophoric variation of the electronic absorption spectrum of these rifamycins with the pH, allows the pK_{a_1} value of the acidic function to be calcd as ~0, while the pK_{a_2} value of the basic function varies from 9.1 to 11.3. These features indicate that these rifamycins are zwitterions, the acidic function being due to the peri (1,8) dihydroxy group, and the basicity to the N atom of the dialkylamino group. The *in vitro* antimicrobial activity of these new rifamycins is reported in Table II.

In comparison with rifamycin SV, most of these derivatives proved to have a better activity against Gram-negative strains. It should be noted that the antibacterial activity decreases with the increase of the length of the aliphatic chain (**1–4**, and **6–7**).

Experimental Section

Pmr spectra were taken on a Varian A60 spectrometer at 60 MHz in $CDCl_3$ (TMS) (0.00 ppm); the mass spectrum was recorded on a Perkin-Elmer 270 spectrometer at 70 eV; electronic absorption spectra were obtained on a Perkin-Elmer Model 4000A spectrometer. Column chromatography was performed with a Merck 0.05- to 0.02-mm silica gel and tlc with HF_{254} Merck.

(5) A. M. Greco, R. Ballotta, and P. Sensi, *Farmaco Ed. Sci.*, **16**, 755 (1961).

(6) G. G. Gallo, L. Chiesa, and P. Sensi, *Anal. Chem.*, **34**, 423 (1962).

4-Dimethylamino-4-desoxyrifamycin SV (1).—To a soln of 3 g (4 mmoles) of rifamycin O in 100 ml of THF, was added 3.5 ml of a MeOH soln (10.5%) of Me_2NH, at room temp. The soln was kept at the same temp overnight and then concd to dryness. The residue was dissolved in 100 ml of EtOAc and washed twice with 20 ml of phosphate buffer pH 7.38, in order to ext rifamycin B. The org layer was dried (Na_2SO_4) and concd under reduced pressure to about 10 ml. The product crystd out and, after chilling (2 hr), was collected, washed with EtOAc, and dried (600 mg). The pmr spectrum shows, together with the common signals of rifamycins, the presence of new signals: $CH_3N^+(H)CH_3$ (2 d, 3.15, 3.22, J = 3.5 Hz, 6 H); 1 arom H (s, 8.82); 2 OH (16.5, 17.6). The two doublets at δ 3.15 and 3.22, after exchange with D_2O, become two singlets: the coupling of Me groups is therefore with a mobile H; this is interpreted as due to the N of Me_2N protonated for internal salt formation.

4-(*N*-Methyl-*N*-benzylamino)-4-desoxyrifamycin SV (6).—To a soln of 4 g (5.3 mmoles) of rifamycin O in 150 ml of THF was added 1.250 g of methylbenzylamine at room temp. After 40 hr, the soln was concd to dryness. The residue was dissolved in 10 ml of $CHCl_3$ and placed on a column of 200 g of silica gel, prewashed with $CHCl_3$, then eluted with a mixt of $CHCl_3$–MeOH 98:2. The first eluate (about 500 ml) was discarded, then the further eluate (about 800 ml) was collected, concd to dryness, dissolved in 20 ml of EtOAc and poured under agitation into 150 ml of hexane. The ppt was collected and dried (1 g). The amorphous yellow products gave a single spot on tlc.

Pmr spectrum showed, together with the common signals of rifamycins, the presence of new signals: $CH_3N^+(H)$ (d, δ 3.24, J = 4.0 Hz, 3 H); $N^+(H)CH_2$ (d, δ 4.42, J = 4.5 Hz, 2 H); C_6H_5 (m, δ 7.7, 5 H); 1 arom. H (s, δ 8.88). Also in this case, the two doublets at 3.24 and 4.42, after exchange with D_2O, become two singlets.

All other products listed in Table I were prepd by the same procedure used for *N*-Me-*N*-benzyl derivative. For simplicity, their preparation has been omitted.

Acknowledgments.—The authors are indebted to Miss G. Carniti for the *in vitro* tests and to Dr. B. Gioia and to Mr. P. Radaelli for the physicochemical determinations.