Table II—Pharmacological Activity of Cinchophen Amides in Mice[a]

Compound	ED_{50} for Analgesic Activity, mg/kg ip	ED_{50} for Pentobarbital Induced Hypnosis, mg/kg ip	LD_{50}[b], mg/kg ip
II	>150	>50[c]	>600
IV	>50	>50	>600
V	>150	>100	>500
VII	>150	>100	>600
VIII	>50	>100	>500
IX	>150	>150	>600
XIII	>100	>100	>600
XIV	>100	>150	>600
XV	>50	>50[c]	>500
XVI	>100	>50	>500
XVII	>150	>100	>600

[a] Each value is the average of four replicate experiments. [b] Taken from the work of Miller and Tainter (12) for comparison. [c] Maximum effect observed.

compared with the inactive 3-nitroaniline isomer (V) suggested that the close proximity of the nitro group to the amino moiety in the phenyl ring is essential for analgesic activity and also that possible electronic interactions exist between them. Of the four closely related analogues (VI–IX), only VIII retained the significant analgesic activity of the parent molecule, while the others showed an opposite effect. This is possibly due to the presence of either a 2-methyl side chain (VI and IX) that undergoes rapid metabolism or to steric hinderance caused by the bulky *tert*-butylamino side chain (VI and VII). The 2-ethyl substituent in VIII could possibly resist metabolic oxidation thereby allowing VIII to reach the blood levels necessary to exhibit a pharmacological effect. Both the *p*-toluidino and *p*-anisidino analogues (XIII and XIV, respectively) showed greater analgesic activity than the parent molecule (I). It is interesting to note that the 2-aminobenzothiazolo analogue (XV) exhibitd a maximum analgesic effect at a dose of 50 mg/kg.

Considerable CNS-depressant activity, as observed by marked reduction in the spontaneous motor activity (SMA) and ptosis, was exhibited with IV but was absent with V, suggesting that the 2-nitroanilino analogue was pharmacologically active while the 3-nitroanilino analogue had no pharmacological effect. The *p*-toluidino analogue (XIII) exhibited CNS-depressant activity while the corresponding *p*-anisidino derivative (XIV) had no effect on the nervous system.

The significant potentiation of pentobarbital-induced hypnosis observed with XV suggests a possible correlation between analgesic activity and the CNS-depressant effect of the 2-aminobenzothiazolo analogue. The 2-aminopyridino analogue (II) also exhibited a maximum effect on the pentobarbital-induced sleeping time in mice (Table II). The presence of the pyridine moiety may be regarded as an essential component for the strong CNS effect of this compound.

REFERENCES

(1) A. Burger, "Medicinal Chemistry," 2nd ed., Interscience New York, N.Y., 1960, p. 349.

(2) J. C. Krantz and C. J. Carr, "The Pharmacological Principle of Medical Practice," 6th ed., Williams and Wilkins, Baltimore, Md., London, 1965, p. 826.

(3) W. D. M. Paton and J. Payne, "Pharmacologic Principles and Practice," Churchill A. Ltd., London, 1969, p. 107.

(4) P. J. Hanzlik, "Action and Uses of the Salicylates and Cinchophen in Medicine," William and Wilkins, Baltimore, Md., London, 1927, p. 3194.

(5) M. T. Bogert and E. M. Ambrahamson, *J. Am. Chem. Soc.*, **4**, 44,826 (1922).

(6) R. Meduedecky, J. Durinda, P. Zakova, and L. Kopacova, *J. Cask. Farm.*, **15**, 291 (1966).

(7) H. Pfitzinger, *J. Prakt., Chem.*, **38**, 582 (1882).

(8) G. Doebner, *Justus Liebigs Ann. Chem.*, **242**, 290 (1887).

(9) A. I. Vogel, "Practical Organic Chemistry," 3rd ed., English Library Book Society, London, 1946, p. 791.

(10) D. Lednicer and L. A. Mitscher, "The Organic Chemistry of Drug Synthesis," Interscience, London, 1977, p. 344.

(11) G. F. Mann and B. C. Saunders, "Practical Organic Chemistry," 5th ed., Longman Green, London, 1957, p. 242.

(12) L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. Med.*, **57**, 261 (1944).

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Structure of the Isonicotinyl Hydrazone of Norethindrone

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Received January 6, 1982, from the *Bureau of Drug Research, Health Protection Branch, Tunney's Pasture, Ottawa, Canada, K1A 0L2.* Accepted for publication August 10, 1982.

Abstract □ The contraceptive steroid norethindrone reacts with isoniazid both *in vivo* and *in vitro* to give the corresponding hydrazone, which exists as *syn* and *anti* (with respect to C-4) isomers. These isomers rapidly interconvert, with the *anti* form predominating in solution. The identification of the isomers was based on an interpretation of ^{1}H- and ^{13}C-NMR spectroscopic data and corroborated by high-performance liquid chromatographic and UV spectrophotometric evidence. ^{1}H- and ^{13}C-NMR spectroscopic data for other derivatives of norethindrone hydrazone are presented and interpreted.

Keyphrases □ Norethindrone—isonicotinyl hydrazone, synthesis, characterization by NMR □ NMR—isonicotinyl hydrazone of norethindrone, characterization, synthesis □ Synthesis—isonicotinyl hydrazone of norethindrone, characterization by NMR

Isoniazid (isonicotinylhydrazine) (I) reacts with ketones and aldehydes under acidic conditions. The usual products are hydrazones, but the reaction with reducing sugars gives 1-glycosyl-2-isonicotinylhydrazines (1). Reactions of this type can take place *in vivo* (2), the pharmacological and toxicological consequences of which are largely unknown. We recently showed that isoniazid reacts with norethindrone (17-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one) (II) to give the hydrazone (III) when they are coadministered orally to the rat (3) and minipig[1]. The product is readily absorbed from the GI tract (4). The analytical procedures involved conversion of norethindrone hydrazone (IV), a metabolite of III (3), to the *p*-methoxybenzaldehyde derivative (V). Some properties of V were described (3). Spectroscopic evidence for the structures of

[1] Unpublished data.

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Table I—Data from ^{1}H-NMR Spectra of II, III, V, and VI [a]

	Solvent	II	IIIa	IIIb	Va	Vb	VIa	VIb
HC=N	VII				8.34	8.34		
	VIII				8.33	8.30		
H-2′,6′	VII		8.75		7.74	7.80		
	VIII		8.72		7.76	7.74		
H-3′,5′	VII		7.74		6.93	7.00		
	VIII		7.76		7.01	7.03		
H-4	VII	5.84	6.31		6.11	6.95	6.11	6.25
	VIII	5.72	6.00	6.51	6.02	6.78	6.02	6.28
OCH_3	VII				3.85	3.85		
	VIII				3.81	3.81		
C≡CH	VII	2.57	2.57		2.56	2.60	2.57	
	VIII	3.29	3.28		3.27	3.29	3.28[b]	3.30[b]
18-CH_3	VII	0.91	0.91		0.91	0.94	0.92	
	VIII	0.79	0.79		0.79	0.79	0.79	
NH	VII						11.24[b]	11.35[b]
	VIII						11.01[b]	11.11[b]

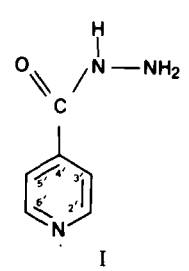
[a] Chemical shifts in ppm downfield from tetramethylsilane. The isomers of V, but not of III or VI, were determined separately. [b] These assignments may be reversed between *a* and *b*.

Table II—Data from ^{13}C-NMR Spectra of II, III, V, and VI [a]

	Solvent	II	IIIa[b]	IIIb[b]	Va[c]	Vb[c]	VIa[d]	VIb[d]
C-3	VII	200.11	—[e]		167.50	164.95	161.09	
	VIII	198.53	—		165.83	163.19	160.82	
C-5	VII	166.71	—		155.90	156.81	154.74	155.59
	VIII	166.77	—		155.47	156.26	154.87	156.26
C-4	VII	124.86	122.50	112.25[f]	122.92	115.57	121.58	111.05
	VIII	123.83	121.43	113.39[f]	122.19	114.78	120.98	110.83
—C≡	VII	87.58	88.08		87.64	87.70	87.64	
	VIII	88.91	88.94		88.97	88.97	88.97	
C-17	VII	79.86	79.86		80.04	79.98	79.89	
	VIII	78.10	78.13		78.16	78.16	78.19	
≡CH	VII	74.28	74.21		74.21	74.21	74.24	
	VIII	74.94	74.88		74.97	74.94	74.94	
C-18	VII	12.75	12.75		12.75	12.75	12.72	
	VIII	12.57	12.57		12.60	12.63	12.63	

[a] Chemical shifts in ppm downfield from tetramethylsilane. The isomers of V, but not of III or VI, were determined separately. [b] The isonicotinic acid hydrazide moiety of III gave signals from C-2′,6′ and C-3′,5′ at 150.31 (150.04) and 122.50 (121.86) ppm, respectively, in VII(VIII); others were not detected. [c] The aryl moiety of Va and Vb gave signals from C-4′, C-2′,6′, C-1′, C-3′,5′, OCH_3, and HC=N at 161.97 (161.52), 130.09 (129.78), 128.20 (127.51), 114.42 (114.42), 55.51 (55.36), and 157.72 (156.84) and at 162.03 (161.49), 130.15 (129.75), 128.08 (127.45), 114.42 (114.39), 55.51 (55.36), and 157.72 (156.69) ppm, respectively, in VII(VIII). [d] The aryl moiety of VI gave double signals from C-1′, C-4′, C-5′, C-2′, C-3′, and C-6′ centered at 145.13 (144.45), 137.81 (136.83), 130.12 (130.17), 129.27 (129.16), 123.83 (123.13), and 116.55 (116.06) ppm, respectively, in VII(VIII). [e] Signals were broad and weak or not detected. [f] Weak signal.

III, V, and the 2,4-dinitrophenylhydrazone of II (VI, prepared as a model compound) is presented here.



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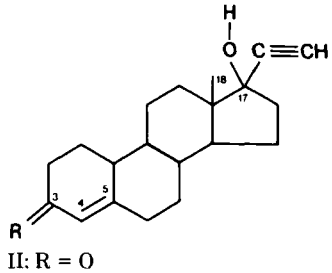


II: R = O
III: R = $C_5H_4NCONHN$
IV: R = H_2NN
V: R = $C_6H_4(OCH_3)CH{=}N{-}N$
VI: R = $C_6H_3(NO_2)_2NHN$

EXPERIMENTAL

Isoniazid[2], *p*-methoxybenzaldehyde[3], hydrazine[4], 2,4-dinitrophenylhydrazine[3], and norethindrone[5] were used to prepare III, V, and VI by general or previously described methods (3). ^{1}H-NMR and broad-band decoupled ^{13}C-NMR spectra were recorded at 80 and 20.1 MHz, respectively, on a Fourier-transform spectrometer[6] at ambient temperature. The deuterium resonance of the solvent, $CDCl_3$[7] (VII) or DMSO-d_6[7] (VIII), provided an internal lock and TMS[8] was the internal reference from which downfield chemical shifts (δ) are expressed in ppm. Interferograms of 4K Fourier-transformed output data points (latterly 8K) and sweepwidths of 1000 and 5000 Hz gave separations in memory addresses of 0.004 and 0.06 (latterly 0.03) ppm for ^{1}H- and ^{13}C-NMR spectra, respectively.

RESULTS AND DISCUSSION

As described previously, hydrazone V is easily separated into isomers Va and Vb, respectively (3). IR spectra of these (KBr pellet) show minor differences in the fingerprint region, but none are helpful in making structural assignments. Their mass spectra are essentially identical, while the UV absorption maximum of Va is at 323 nm and Vb is at 315 nm (3).

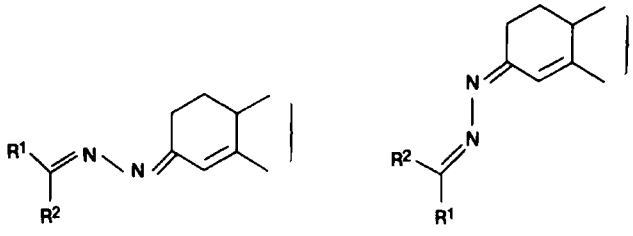
^{1}H-NMR spectroscopic data for II, III, Va, Vb, and VI are presented in Table I. Two isomers (~1:1, VIa–VIb) could be detected in the spectra of VI and in the spectrum of III determined in DMSO-d_6 (~2:1, IIIa–IIIb). Signals of isomers have closely similar chemical shifts except for the signal assigned to H-4, which has a width at half-height ($w_{1/2}$) of ~5 Hz in each case. The chemical shifts and widths of these signals are appropriate for Δ^4 rather than Δ^5 structures (5). ^{1}H-NMR data for *syn* and *anti* (with respect to C-4) oximes of some 3 keto-Δ^4 steroids show that H-4 appears at ~6.5 and 5.9 ppm, respectively (6), suggesting that the *a* forms are the *anti* isomers.

Data from the ^{13}C-NMR spectra are presented in Table II. It is beyond our present scope to assign all of the signals, especially the 14 signal (steroidal) appearing between ~20 and 50 ppm, but assignments of the remainder have been made by comparison with compiled data (7). Limited quantities of III, the presence of both isomers in DMSO-d_6, and

[2] Sigma Chemical Co., St. Louis, Mo.
[3] BDH Chemicals Ltd., Poole, England.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] Supplied by G. D. Searle, Chicago, Ill.
[6] Bruker WP80; Bruker Spectrospin (Canada) Ltd., Mississauga, Ont.
[7] Merck Sharpe & Dohme (Canada) Ltd., Montréal, Québec.
[8] Stohler Isotope Chemicals, Montréal, Québec.

its low solubility in $CDCl_3$ prevented us from detecting all of the signals. It is also possible that the rates of interconversion of the two forms are such that while the proton chemical shifts are time-averaged in $CDCl_3$, although distinct in DMSO-d_6, certain of the ^{13}C-NMR signals are broadened by these processes and hence obscured (8). Variable temperature studies that might answer these questions are beyond our present capabilities. The ^{13}C-NMR spectra showed differences at C-3, C-4, and C-5 of ~2.5 and 7 ppm downfield and 1 ppm upfield, respectively, for V*a* in comparison with V*b*. Comparable differences were also found between the unseparated forms of III and VI and so have been assigned to *a* and *b* isomers in Tables I and II by comparison with V*a* and V*b* data. The resonances of carbons *anti* to hydrazones and oximes appear 6–12 ppm downfield from the positions of the corresponding *syn* carbons (9), again in agreement with assignment of the *anti* configuration to the *a* forms.

In further support, the order of elution (V*a* followed by V*b*) on high-performance liquid chromatography (HPLC) (3) is compatible with this assignment (6), and the UV data also suggest that the conjugated double bonds are more extended in V*a* than in V*b* (10, 11). Thus, the structures depicted are initially proposed for this series of compounds (the most stable rotational conformation about the N—N bond is shown).



V*a*,V*a*′ V*b*,V*b*′

V*a*,V*b*: R^1 = *p*-$(OCH_3)C_6H_4$, R^2 = H
V*a*′,V*b*′: R^1 = H, R^2 = *p*-$(OCH_3)C_6H_4$

A particularly interesting feature of the spectra of VI was that the aromatic carbon-13 signals were all doubled (except for C-3′ and C-5′ in $CDCl_3$). The signals, separated by 0.1–0.2 ppm, presumably arise from the *syn* and *anti* isomers, and were assigned (Table II) by comparison with the spectrum of the 2,4-dinitrophenylhydrazone of cyclohexanone in which they are not, of course, doubled. The additional possibility in azine V of *anti*/*syn* isomerism about the aldimino double bond (HC=N) leads to alternative structures V*a*′ and V*b*′, which are expected to have very similar NMR and UV absorption properties to those of V*a* and V*b*, respectively. It does not seem that mixtures are present, and it is very unlikely that the two forms of V are V*a* with V*b*′ or V*a*′ with V*b*, as neither the ^1H-NMR nor the ^{13}C-NMR shifts of the HC=N function differ between isomers (Tables I and II). The stable *anti* aldimino configuration (V*a* and V*b*) is favored.

It is evident that hydrazine-based derivatives of norethindrone can be produced *in vivo* by interaction with isoniazid. We have found that the metabolic disposition of the steroid is thereby altered[1]. Other pharmacologically important steroids and possibly other hydrazine-derived drugs may undergo similar interactions. *Syn* and *anti* isomers of norethindrone hydrazones arise and can be identified. They undergo rapid interconversion in some cases, but may be separated in others. Whether tissue enzymes, which further metabolize the hydrazones (3), are selective for *syn*/*anti* isomers remains to be determined. If such selectivity were to occur, one might expect that the rate of metabolism of various hydrazones would be dependent, *inter alia*, on the relative degree of interconvertibility of the isomers.

REFERENCES

(1) K. Bailey and A. G. Butterfield, *Can. J. Chem.*, **59**, 641 (1981).

(2) J. P. O'Donnell, W. J. Proveaux, and J. K. H. Ma, *J. Pharm. Sci.*, **68**, 1524 (1979).

(3) H. Watanabe, J. A. Menzies, N. Jordan, and J. C. K. Loo, *Res. Commun. Chem. Path. Pharmacol.*, **31**, 435 (1981).

(4) H. Watanabe, J. A. Menzies, and J. C. K. Loo, *Experientia*, **37**, 883 (1981).

(5) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964, pp. 87–90.

(6) M. Patthy and E. Tomori, *J. Chromatogr.*, **191**, 145 (1980).

(7) E. Breitmaier, G. Haas, and W. Voelter, "Atlas of Carbon-13 NMR Data," Vol. 2, Heyden, Philadelphia, Pa., 1979, entries 2673–84.

(8) F. W. Wehrli and T. Wirthlin, "Interpretation of Carbon-13 NMR Spectra," Heyden, New York, N.Y., 1976, pp. 197–215.

(9) C. A. Bunnell and P. L. Fuchs, *J. Org. Chem.*, **42**, 2614 (1977).

(10) J. R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds," Prentice-Hall, Englewood Cliffs, N.J., 1965, pp. 19, 20.

(11) D. J. Cram and G. S. Hammond, "Organic Chemistry," McGraw-Hill, New York, N.Y., 1959, p. 618.

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Effect of the Nonionic Surfactant Poloxamer 338 on the Fate and Deposition of Polystyrene Microspheres Following Intravenous Administration

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Received December 11, 1981, from the **Department of Pharmaceutics, The Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark*, and the ‡*Department of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.* Accepted for publication August 25, 1982.

Abstract □ The blood clearance and organ deposition of polystyrene microspheres in the rabbit following intravenous injection has been investigated using the technique of gamma scintigraphy, blood and organ level measurements, and histology. Uncoated microspheres of 1.27-μm diameter were cleared rapidly from the blood and were taken up primarily by the reticuloendothelial system in the liver. Coating of the microspheres with the nonionic surface-active agent poloxamer 338 reduced the uptake in the liver and gave a corresponding increase in the lungs.

Keyphrases □ Microspheres, polystyrene—effect of nonionic surfactants on blood clearance and deposition, intravenous administration □ Nonionic surfactants—effect on blood clearance and deposition of polystyrene microspheres, intravenous administration □ Deposition, tissue—polystyrene microspheres following intravenous administration, effect of nonionic surfactants □ Blood clearance—polystyrene microspheres following intravenous administration, effect of nonionic surfactants

Colloidal systems such as liposomes, microspheres, nanospheres, and emulsions have been investigated as potential drug-targeting devices (1–4). The fate of such particles in the body following administration is deter-

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