valent copper is fructose > glucose > galactose. Joslyn and Miller (11) reported that I was more effective than sucrose and dextrose in stabilizing ascorbic acid solutions against oxidative degradation. These authors concluded that no simple rate law governed the oxidation of ascorbic acid in the presence of sugars. They also observed that the temperature coefficients of the oxidation in the presence or absence of the sugars were similar. This observation would seem to indicate that complex formation was not occurring since the temperature coefficients would be expected to differ. Ashida (12) also reported that methylene blue was more easily reduced by a ketose such as fructose than by an aldose such as glucose.

The data and speculations serve as a stimulus for additional research to establish a mechanism for the stabilization observed. It will be of interest to learn if the redox potential of I is similar to that for II. Such similarity is anticipated because of the structural relationship between the portions of the two molecules where oxidation reactions have been shown to occur. The redox potentials of the other polyols are anticipated to differ significantly.

REFERENCES

(1) L. Velluz, A. Petit, M. Pesez, and R. Berret, *Bull Soc. Chim. France*, **1947**, 123.

(2) P. T. Herzig and M. Ehrenstein, *J. Org. Chem.*, **16**, 1050 (1951).

(3) H. L. Mason, C. S. Myers, and E. C. Kendall, *J. Biol. Chem.*, **116**, 267 (1936).

(4) H. L. Mason, *Proc. Staff Meetings Mayo Clinic*, **13**, 235 (1938).

(5) N. L. Wendler and R. P. Graber, *Chem. Ind. (London)*, **1956**, 549.

(6) D. E. Guttman and P. D. Meister, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 773 (1958).

(7) E. Caspi, W. Schmid, and T. A. Wittstruck, *Tetrahedron*, **16**, 271 (1961).

(8) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **21**, 2001 (1963).

(9) J. W. Mauger, A. N. Paruta, and R. J. Gerraughty, *J. Pharm. Sci.*, **58**, 574 (1969).

(10) M.Pd. Singh, B. Krishna, and S. Ghosh, *Z. Phyzik. Chem. (Leipzig)*, **205**, 285 (1956).

(11) M. A. Joslyn and J. Miller, *Food Res.*, **14**, 340 (1949).

(12) K. Ashida, *Mem. Inst. Sci. Ind. Res., Osaka Univ.*, **10**, 205 (1953).

Cinchophen Analogues as Potential CNS Agents

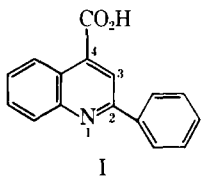
A. KAR

Received October 20, 1981, from the *Department of Pharmaceutical Chemistry, University of Nigeria, Nsukka, Nigeria.* Accepted for publication June 1, 1982.

Abstract □ Several amides of cinchophen were prepared and evaluated as CNS agents. Compounds III, VII, XII, XIII, and XIV exhibited analgesic activity while I, III, and XIV acted as CNS depressants.

Keyphrases □ Cinchophen—amide analogue synthesis, evaluation of CNS activity in mice □ Analgesics—potential, amide analogues of cinchophen, evaluation of CNS activity in mice □ Central nervous system agents—amide analogues of cinchophen, evaluation of CNS activity in mice

Cinchophen (I) was formerly used for the treatment of gout (1, 2); however, it was withdrawn from the market because of its toxic effects (3–5). It was concluded that the quinoline ring, a carboxylic acid group in the C-3 or C-4 position, and an aryl residue at C-2 were essential for the physiological action of cinchophen (6). Many structural modifications of cinchophen have been reported [*i.e.*, neocinchophen, 2-phenyl-3-hydroxycinchonic acid, hexophan, and 2-phenyl-4-hydroxyacetyl-6-methoxyquinoline (1)]. Reversal of the phenyl and carboxyl groups afforded an inactive compound (I).



I

In this study 16 amides of I were synthesized and evaluated as CNS agents. Cinchophen has been synthesized either by the condensation of acetophenone with isatic acid in alcoholic potassium hydroxide solution (7) or by heating pyruvic acid with either aniline and benzaldehyde or with benzylidene aniline in absolute ethanol (8). In the present study, I was prepared by the condensation of pyruvic acid with aniline and benzaldehyde (9, 10). The desired amides were subsequently synthesized by treating the corresponding acid chloride with the appropriate amine.

EXPERIMENTAL

Synthesis—The amides II–XVII were prepared (11) by vigorously shaking for 30 min at room temperature a mixture of the appropriate amine (0.002 mole), a 10–15% molar excess of the acid chloride of cinchophen, and 10 ml of 10% aqueous sodium hydroxide solution. The resulting solid material was removed by filtration and purified, as indicated in Table I.

Analysis and Spectral Data—Melting points were determined in open capillary tubes in an electrothermal apparatus and are uncorrected. NMR[1] spectra were obtained in deuterated acetone using tetramethylsilane as the internal standard. The mass spectra[2] of the various compounds were determined. Microanalyses were within ±0.3% of the theoretical values (Table I).

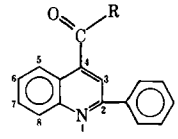
Pharmacological Evaluation—The hot plate method was employed to evaluate the analgesic activity of the amides in mice. The CNS-depressant activity was studied *in vivo* by noting the effect of the amides on spontaneous motor activity, ptosis, and pentobarbital-induced hypnosis. Swiss albino mice of both sexes, weighing 20–30 g, were used; solutions of normal saline or 3% (w/v) aqueous polysorbate 80 were given to the untreated and vehicle controls, respectively. Cinchophen (50 or 100 mg/kg), pentobarbital sodium (40 mg/kg), and morphine hydrochloride (4 mg/kg) were used as reference drugs to evaluate the activity of the amides.

Analgesic Activity—Analgesic activity was measured by the hot plate method at 53 ± 2°. The normal reaction time (pain threshold) for each mouse was determined by placing the mouse on the hot plate and noting the amount of time (≤5 sec) required for the mouse to leave the hot plate platform. The pain threshold was measured 30 min postinjection and at hourly intervals for 4 hr.

[1] Perkin-Elmer R-32 spectrometer.
[2] VG-Micromass 16F (with a VG system 2000 computer).

0022-3549/83/0900-1082$01.00/0

Table I—Physical Properties of Cinchophen Amides



Compound	R	Yield,[a] %	mp, °	Formula	Analysis, % Calc.	Found	IR Spectra (KBr), cm^{-1}	^1H-NMR Spectra (acetone d_6), δ
II	2-Aminopyridino	85	119–121	$C_{21}H_{15}N_3O$	C 77.5 H 4.61 N 12.92	77.72 4.60 12.89	3300–3400 (m,—NH), 1660 (w,C=O), 1500 (aromatic), 1340 (s,aryl NH_2), 770 (s, pyridine, 2-mono-subst.)	8.7 (s,1,C—3); 8.3 (d, 4, C_5—C_8); 8.1 (s,1,—NH, C—9); 7.4 (d,5, C—2 phenyl)
III	2-Aminopyrimidino	21	183	$C_{20}H_{14}N_4O$	C 73.61 H 4.29 N 17.17	73.57 4.30 17.21	3400 (m,—NH), 1620 (m, C=O), 1320 (w, aryl-NH_2)	8.8 (s,1,C—3); 8.3 (d,4,C_5—C_8); 8.15 (s,1,—NH, C—9); 7.4 (d,5, C—2 phenyl)
IV	2-Nitroanilino	90	195–197	$C_{22}H_{15}N_3O_3$	C 71.54 H 4.06 N 11.38	71.22 4.03 11.35	3300–3400 (m,—NH), 1660 (w, C=O), 1600 (w, phenyl), 1320 (m, nitro), 700 (monosubst. aryl CH)	8.75 (s,1,C—3); 8.45 (d,4,C_5—C_8); 8.1 (s,1,—NH, C—9); 7.7 (d,4, C—3′ to C—6′); 7.4 (d,5, C—2 phenyl)
V	3-Nitroanilino	62	121–123	$C_{22}H_{15}N_3O_3$	C 71.54 H 4.06 N 11.38	71.64 4.08 11.40	3400 (m,—NH), 1660 (w, C=O), 1600 (w, phenyl), 1330 (m, NO_2), 720 (monosubst. aryl CH)	8.80 (s,1,C—3); 8.45 (d,4,C_5—C_8); 8.15 (s,1,—NH, C—9); 7.65 (d,4, C—3′ to C—6′); 7.4 (d,5,C—2 phenyl)
VI	2-Methyl-6-*tert*-butylanilino	85	214–216	$C_{27}H_{26}N_2O$	C 82.23 H 6.59 N 7.10	82.02 6.61 7.12	3400 (w,—NH), 1670 (m,C=O), 1600 (m,phenyl), 1340 (s, aryl —NH_2)	8.75 (s,1,C—3); 8.45 (d,4,C_5—C_8); 7.3 (d,5, C—2 phenyl), 2.1 (d,3, C—9,2—CH_3): 1.4 (t,9, C—9, 6 *tert*-butyl)
VII	2-Ethyl-6-*sec*-butylanilino	82	113	$C_{28}H_{28}N_2O$	C 82.35 H 6.86 N 6.86	82.17 6.88 6.84	3400 (m,—NH), 1670 (m,C=O), 1340 (s, aryl *sec* amino group), 700 (s, mono-subst. aryl CH)	8.7 (s,1,C—3); 8.1 (s,1,—NH); 7.7 (d,3,C—9, C—3′ to C—5′); 7.3 (d,5, C—2 phenyl); 2.8–2.4 (d, 5, C—9, —C_2H_5); 2.0 (t,9, C—9, 6 *sec*-butyl)
VIII	2-Ethyl-6-isopropylanilino	75	171–173	$C_{27}H_{26}N_2O$	C 82.23 H 6.59 N 7.10	82.31 6.57 7.09	3400 (m,—NH), 1670 (m,C=O), 1340 (s, aryl *sec* amino group)	8.7 (s,1,C—3); 8.4 (d,4, C—5 to C—8); 7.7 (d,3, C—9, C—3′ to C—5′); 7.45 (d,5, C—2 phenyl); 3.0 (s,1,C—6); 1.4–1.0 (d,6, C—9 isopropyl)
IX	2-Methyl-6-isopropylanilino	92	315–317	$C_{26}H_{24}N_2O$	C 82.10 H 6.31 N 7.37	81.89 6.29 7.34	3400 (m,—NH), 1500 (s, aromatic), 1380 (s, isopropyl)	8.7 (s,1,C—3); 8.2 (d,4,C_5—C_8); 7.8 (d, 3,C—3′ to C—5′); 7.4 (d, 5, C—2 phenyl)
X	Morpholino	42	199–201	$C_{20}H_{18}N_2O$	C 79.47 H 5.96 N 9.27	79.29 5.98 9.29	3420 (m,—NH), 1660 (m,C=O), 1510 (m, aromatic)	8.7 (s,1,C—3); 8.1 (d,4,C—5 to C—8); 7.6 (d,3,C—3′ to C—5′); 7.2 (d,5,C—2 phenyl)
XI	Pyrrolidino	25	195	$C_{20}H_{18}N_2O$	C 79.47 H 5.96 N 9.27	79.55 5.94 9.26	1670 (m,C=O), 1500 (m, aromatic), 720 (mono-subst. aryl CH)	8.75 (s,1,C—3); 8.1 (d,4,C_5—C_8); 7.9 (d,8,C—2′ to C—5′); 7.1 (d, 5,C—2 phenyl)
XII	Piperidino	38	189	$C_{21}H_{20}N_2O$	C 79.74 H 6.32 N 8.86	79.68 6.34 8.88	1640 (m,C=O), 1500 (m, aromatic), 730 (monosubst. aryl CH)	8.7 (s,1,C—3); 8.2 (d,4,C_5—C_8); 7.85 (d,10,C—2′ to C—6′); 7.1 (d,5,C—2 phenyl)
XIII	*p*-Toluidino	92	90–91	$C_{22}H_{18}N_2O$	C 80.98 H 5.52 N 8.58	81.10 5.53 8.57	3400(m,—NH), 1670 (w,C=O), 1620 (m, amide), 1600 (m, phenyl), 1500 (m, aromatic)	8.7 (s,1,C—3); 8.5 (d,4,C_5—C_8) 7.7 (d,4,C—2′,3′,5′ & 6′); 7.4 (d,5, C—2 phenyl); 2.1 (d,3, C—4′ methyl)
XIV	*p*-Anisidino	85	299–301	$C_{22}H_{18}N_2O_2$	C 77.19 H 5.26 N 8.18	77.27 5.25 8.20	3400 (m,—NH), 1670 (w,C=O) 1620 (m, amide), 1172 (m,—OCH_3), 700 (s, monosubst. aryl CH)	8.75 (s,1,C—3); 8.5 (d,4,C_5—C_8); 7.7 (d,4,C—2′,C—3′,C—5′ & C—6′); 6.65 (d,3, C—4′—OCH_3);
XV	2-Aminobenzo thiazolyl	88	110	$C_{23}H_{15}N_3OS$	C 72.44 H 3.93 N 11.02 S 8.39	72.14 3.92 10.99 8.41	3400 (m,—NH), 1650 (m,C=O), 1110 (m, aryl), 700 (s, monosubst. aryl CH)	8.70 (s,1,C—3); 8.5 (d,4,C—5 to C—8); 8.0 (s,1,—NH at C—9); 7.4 (d,5, C—2 phenyl); 7.1 (d,4 C—3′ to C—6′)
XVI	Diphenylamino	91	56–58	$C_{28}H_{20}N_2O$	C 84.00 H 5.00 N 7.00	83.88 5.02 7.02	1680–1630 (s, *tert* amide), 1660 (m,C=O), 1600 (w, phenyl), 1500 (s, aromatic)	8.3 (d,4,C_5—C_8); 7.4 (d,5 C—2 phenyl); 4.2 (d,10,C—9)
XVII	5-Nitroisatoic anhydride	85	248–250	$C_{24}H_{13}N_3O_6$	C 65.60 H 2.96 N 9.56	65.52 2.96 9.54	3400–3300 (m,—NH), 1650 (w,C=O), 1600 (w, phenyl), 1320 (m, nitro), 700 (monosubst. aryl CH)	8.25 (d,4,C_5—C_8); 7.4 (d,5,C—2 phenyl); 3.7 (d,3,C—9 aryl);

[a] All compounds were recrystallized from 95% ethanol except for XVII which was recrystallized from water.

Spontaneous Motor Activity—The increase in SMA was measured using an activity cage[3] 30 min postinjection and at hourly intervals for 4 hr. Any change in the activity levels was noted.

Pentobarbital-Induced Hypnosis—Fifteen minutes postinjection, all animals were treated with 40 mg/kg of pentobarbital sodium. The time to the loss of the righting reflex was noted for each mouse; the mice were then placed on their backs. When the animals regained their righting reflex (*i.e.*, starting moving around the cage) the time was again noted, and the duration of the pentobarbital-induced hypnosis was calculated.

[3] Activity Cage 7401, Ugo Basile, Biological Research Apparatus, Comerio-(va)-Italy.

RESULTS AND DISCUSSION

Of the 16 compounds synthesized, 12 were evaluated as CNS agents. The significant analgesic activity of the 2-nitroanilino analogue (IV)

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).

ACKNOWLEDGMENTS

The author is deeply indebted to E. C. Onyeji and C. N. Obijiofor for their valuable assistance in the pharmacological screening. In addition, the author thanks George McDonnough and Alan Passmoor of Chelsea College, University of London, for the spectral data.

Structure of the Isonicotinyl Hydrazone of Norethindrone

KEITH BAILEY ˣ, ALLAN MENZIES, and HISAKO WATANABE

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.

0022-3549/83/0900-1084$01.00/0