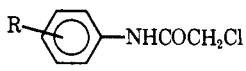
Experimental Section

Melting points were determined in capillary tubes in oil bath and are uncorr. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Ir spectra were determined in KBr by El-Nasr Company, U.A.R., using a Beckmann spectrometer IR-20. The spectra were carried out using high resoln in the range of 1800–1500 cm^{-1}. The mol wt was determined by the Rast method.

N-Chloroacetylanilines.—To 18.6 g (0.2 mole) of redistd $PhNH_2$ in 15.6 (0.2 mole) of pyridine, and 200 ml of PhH, 22.6 g (0.2 mole) of $ClCOCH_2Cl$ in 100 ml of PhH was added dropwise. The reaction was carried out in an ice–water bath with stirring for 1 hr. The solvents were removed under reduced pressure and the oily residue was dissolved in 50 ml of ETOH and poured into 100 ml of cold H_2O previously acidified with 5 ml of 1 *N* HCl. The sepd *N*-chloroacetyl aniline was crystd from 40% ETOH; yield 26 g (76%), mp 137–138° as reported.[7]

The data for the other 2-chloro-2′-(or 4′-)substituted acetanilides are given in Table V.

TABLE V



R	Yield, %	Mp, °C	Ref
2-OH	30	136	*a*
4-OH	23	142	*b*
2-OEt	84	72	*c*
4-OEt	84	142–144	*d*
2-Me	80	112	*e*
4-Me	80	158–160	*f*
2-COOEt	75	74	*g*
4-COOEt	75	116	*h*

[a] W. A. Jacobs, M. Heidelberger, and I. P. Rolf, *J. Amer. Chem. Soc.*, **41**, 458 (1919). [b] W. A. Jacobs and M. Heidelberger, *ibid.*, **39**, 1442 (1917). [c] M. Heidelberger and W. A. Hacobs, *ibid.*, **41**, 1452 (1919). [d] A. Bistrzycki and F. Ulffers, *Ber.*, **31**, 2790 (1898). [e] W. Abenius, *J. Prakt. Chem.*, **38**, 299 (1888). [f] H. Eckenroth and A. Donner, *Ber.*, **23**, 3288 (1890). [g] W. A. Jacobs, M. Heidelberger and I. P. Rolf, *J. Amer. Chem. Soc.*, **41**, 469 (1919). [h] G. Sanna and M. Granata, *Chem. Zentraibl.*, **108**, 3313 (1937); *Chem. Abstr.*, **33**, 5827 (1939).

1-Carbethoxypiperazine was obtained in 70% yield, bp 237°;[8] pK_a = 8.04 ± 0.04, potentiometrically at 30° following a procedure modeled upon the method of Albert and Serjeant.[9] **1-Ethylpiperazine·2HCl** was obtained in 80% yield.[8]

Piperazine and Ephedrine Derivatives (Tables I–III). (A) 1,4-Bis[(2- or 4-substituted)phenylcarbamoylmethyl]piperazine (3–10).—To a soln of 0.01 mole of 2-chloro-2′-(or 4′-) substituted acetanilide in 30 ml of EtOH was added 1.0 g of anhyd Na_2CO_3 followed by a soln of 0.97 g (0.005 mole) of piperazine in EtOH. The mixt was stirred while refluxing for 12 hr. The alcohol was distd off and 50 ml of H_2O was added to the residue. A solidifying oily layer sepd which was filtered, washed (H_2O), and recrystd twice from propylene glycol.

(B) 1-Carbethoxy-4-[(2- or 4-substituted)phenylcarbamoylmethyl]piperazine (11–18).—These compds were obtained as in A, using 0.01 mole of 2-chloro-2′-(or 4′-) substituted acetanilide (1.58 g), 0.01 mole of 1-carbethoxypiperazine, and 0.5 g of anhyd Na_2CO_3. The products were recrystd from 50% EtOH.

(C) 1-Ethyl-4-[(2- or 4-substituted)phenylcarbamoylmethyl]-piperazine (19–25).—To a soln of 0.46 g (0.02 g-atom) of Na in 30 ml of EtOH, 1.87 g (0.01 mole) of ethylpiperazine·2HCl was added, and the mixt was refluxed for 1 hr. To the cooled mixt was added 0.5 g of anhyd Na_2CO_3 and 0.01 mole of 2-chloro-2′-(or -4′-)substituted acetanilide. The mixt was refluxed with stirring for 12 hr. After removal of EtOH, 50 ml of H_2O was added to dissolve the oily residue. The aq soln was extd 3 times with 20-ml portions of Et_2O. The Et_2O soln was dried (Na_2SO_4), filtered, and evapd. The HCl salts were hygroscopic. For analysis, the dipicrates were prepd and recrystd from EtOH.

(7) Th. Zincke and O. Kegel, *Ber.*, **23**, 243 (1890).

(8) T. S. Moore, M. Boyle, and V. M. Thorn, *J. Chem. Soc.*, 39 (1929).

(9) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen & Co., Ltd., London, 1962.

(D) 2-(2-Hydroxy-1,N-dimethyl-2-phenylethylamino)-2′- (or 4′-)substituted Acetanilide (26–32).—To a soln of 0.23 g (0.01 g-atom) of Na in 30 ml of EtOH, 2 g (0.01 mole) of ephedrine·HCl was added and the mixt was refluxed for 1 hr. To the cooled mixt were added 0.5 g of anhyd Na_2CO_3 and 0.01 mole of 2-chloro-2′-(or 4′-)subst acetanilide. The mixt was refluxed with stirring for 12 hr. After distn of EtOH, the residue was extd 3 times with 20-ml portions of Et_2O. The Et_2O soln was extd exhaustively with 1 *N* HCl. The combined acidic solns were neutralized with concd NH_4OH and the milky white colloidal soln was warmed and then left overnight. The residue was recrystd from 60% EtOH.

Test for Corneal Anesthesia.—The HCl salt solns of the compds were prepared in normal saline, and 0.25 ml was placed in the rabbit's cornea. Three concns for each compd in the range 0.5–4% of the base were tested on both eyes. The sol HCl salts were found to have a pH 3.8–4.0; an attempt to raise the pH to the neutral side resulted in the pptn of the bases. Corneal reflex detn was made at 2-min intervals following the start of anesthesia.

Acknowledgment.—The authors are indebted to Dr. A. M. Afifi for invaluable help in the pharmacological part of the present investigation.

A Stable, Biologically Active Indoxyl

SOUHEIL E. SAHEB* AND MAKHLUF J. HADDADIN

Department of Pharmaceutical Chemistry and the Department of Chemistry, American University of Beirut, Beirut, Lebanon

Received September 14, 1970

While many indole derivatives are known to be biologically active[1] little is known about the activity of indoxyls (indol-3-ol). The scarcity of information[2] on such compounds is most probably due to their instability as they tend, unless disubstituted at position 2, to undergo oxidation and or dimerization.[3]

The purpose of this work was to synthesize stable indoxyl derivatives and test them for biological activity. In a previous report,[4] it was shown that *o*-nitrobenzaldehyde reacted with keto steroids to yield steroidal indoxyls. The effect of the steroidal nucleus on the (potential) biological activity of the indoxyl derivatives rendered steroidal indoxyls unsuitable for our purpose.

The reaction of *o*-nitrobenzaldehyde with some cyclic ketones (cyclopentanone, cyclohexanone) proceeded vigorously and lead to intractable tars, presumably *via* unstable indoxyls. Mechanistic consideration indicated that a 1,4-cyclodione would lead to stable indoxyl derivatives, where C_2 of the indoxyl moiety will constitute the α position of an α,β-unsaturated ketone. It was envisaged that such a conjugation would stabilize the indoxyl structure. Indeed, the reaction of *o*-nitrobenzaldehyde with 1,4-cyclohexanedione in 5% methanolic KOH gave Ia and Ib in 32 and 20% yield, resp. The products were separated by chromatography.

(1) W. M. McIsaacs and R. T. Harris, *Advan. Pharmacol.*, **6**, 247 (1968); A. Hoffman, *Med. Res. Ser.*, **2**, 221 (1968).

(2) R. G. Taborsky, *Int. J. Neuropharmacol.*, **7**, 483 (1968); A. Pelczarska, *Arch. Immunol. Ther. Exp.*, **17**, 118 (1969).

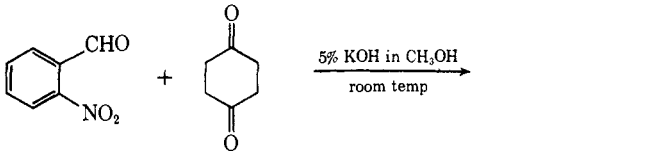
(3) (a) A. Hassner and M. J. Haddadin, *J. Org. Chem.*, **28**, 224 (1963). (b) A. Albert, "Heterocyclic Chemistry," Athlone Press, London, 2nd ed, 1968, pp 209 and 241. (c) R. M. Acheson, "An Introduction to the Chemistry of Heterocyclic Compounds," Interscience, New York, N. Y., 2nd ed, 1967, p 163. (d) Reference 3c, p 155.

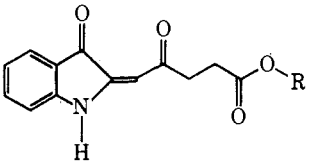
(4) A. Hassner, M. J. Haddadin, and P. Catsoulacos, *J. Org. Chem.*, **31**, 1363 (1966).

TABLE I

		Ia			Ib		
		Acid	Base	Neutral	Acid	Base	Neutral
$\lambda_{max\ (aq)}$	uv	277	277	277	277	277	277
	vis	474	478	474	470	470	470
$\epsilon_{max\ (aq)}$	uv	2.87	2.72	2.87×10^4	1.05	1.84	1.8×10^4
	vis	6.2	5.6	6.2×10^3	4.09	3.28	4.09×10^3

The structure of the orange-red Ia was established by the following data: in addition to an ir band at 1725 cm^{-1}, product Ia showed strong absorption at 3350 (NH), 1650 (C=O), and 1610 cm^{-1} (NC=C). Its nmr spectrum displayed a multiplet at τ 3.35 and 2.8

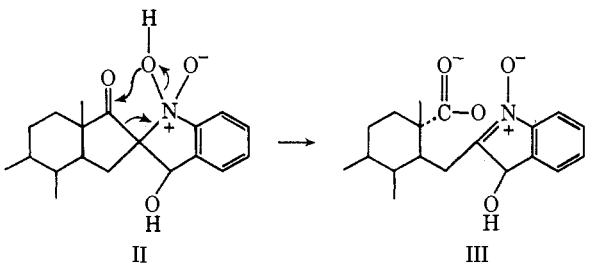




Ia, R = CH_3
Ib, R = H

(4 H), 2 singlets at 4.15 (I H) and 6.6 (3 H), and a quartet at 7.5 (4 H). Its fragmentation pattern in the mass spectrum is consistent with the assigned structure (see Experimental Section). Reduction of Ia with $NaBH_4$ in MeOH and subsequent treatment of the acidified solution with *p*-dimethylaminobenzaldehyde gave an intense red color indicative of an indole structure;[3d] Ia gave a 2,4-dinitrophenylhydrazone.

The formation of Ia sheds more light on the mechanism of the reaction of *o*-nitrobenzaldehyde with ketones to yield indoxyls. The proposed mechanism of this reaction, using keto steroids, involves the formation of intermediate II which undergoes an intramolecular rearrangement to give intermediate III.[4] Partial evi-



II III

dence in favor of an intramolecular nucleophilic attack was advanced on the basis that the reaction in anhyd MeOH, gave a steroidal acid instead of its Me ester. The formation of Me ester Ia clearly indicates that an intermolecular attack cannot be excluded. Moreover, Ia was the sole product when the reaction was run in abs MeOH.

Examination of a molecular model of the proposed intermediate II showed that the C_{17}=O group is fairly crowded by the disubstituted C_{13} and C_{16} and therefore unavailable for an attack by MeO^-. Such steric effects are substantially reduced or absent in the case of 1,4-cyclohexanedione. Explanation of these results in terms of steric hindrance is further supported by the isolation of acid Ib as the only product when the reaction was performed in *tert*-BuOH.

Biological Results.—Compound Ia could not be tested for biological activity due to its complete insolubility in H_2O. On the other hand, the water soluble Ib, when added in a final concentration of 0.5 mg/ml to the bath of a properly prepared rat uterus,[5] showed a weak inhibition of the contraction initiated by serotonin (0.2 mg/ml). In the cat, iv injection of Ib at 0.1 g/kg lowered the blood pressure, increased the depth of respiration, and provoked a spontaneous ear twitch. Repeated exposure of the same cat to Ib gave a gradually diminishing response. These observations are comparable to the effects observed following repeated injections of serotonin.

Furthermore, compound Ia inhibited the growth of *Sarsina lutea* (G + ve) at 37 μg/ml, but did not inhibit the growth of *Escherichia coli*.[6]

Experimental Section

Ir spectra were taken in KBr on a Perkin-Elmer 237A. Uv spectra were measured in water using a Perkin-Elmer 202, nmr spectra in $CDCl_3$. Melting points are corrected.

2-(Methyl 3′-oxopent-5′-enoate)indoxyl (Ia) and 2-(3′-Oxopent-5′-enoic acid)indoxyl (Ib).—A soln of *o*-nitrobenzaldehyde (1.51 g, 0.01 mole) in 90% MeOH (6 ml) contg 5% KOH was added to a soln of 1,4-cyclohexanedione in 90% MeOH (6 ml). The mixt, which instantaneously developed a succession of colors (yellow-blue-red), was allowed to stand at room temp for 3 hr. The acidified soln (5 ml of concd HCl) was left to stand overnight and the resulting brown-red residue was filtered off, air-dried, and dissolved in the least amt of $CHCl_3$. This soln was put on a silica column and eluted with $CHCl_3$. Ia was obtained as an orange-red solid, mp 199-200° (recrystd from $CHCl_3$). Its 2,4-dinitrophenylhydrazone melted at 172-175°. The soln of Ia in alcoholic base turned blue in 1 min. *Anal.* Calcd for $C_{14}H_{13}NO_4$: C, 63.41; H, 4.8; N, 5.68. Found: C, 63.37; H, 4.92; N, 5.19. The mass spectrum of Ia showed peaks at 258, 200, 172, 144, and 160.

The acid Ib remained on the top of the column and was eluted with MeOH. It melted at 155-156°, had the same nmr pattern as Ia except for the singlet Me absorption at τ 6.6 and was converted up to 50% into Ia by standing everynight in MeOH.

Acknowledgment.—We should like to thank Miss Sonia Shamlian for her able assistance, Professor Walter T. Smith of the University of Kentucky for the mass spectrum, and Dr. Nabil Banna for his advice on the biological experiments. Financial support from the Medical Research Committee of the American University of Beirut is gratefully acknowledged.

(5) R. A. Turner, "Screening Methods in Pharmacology," Academic Press, New York, N. Y., 1965, p 127.

(6) F. Kavanagh, "Analytical Microbiology," Academic Press, New York, N. Y., 1963, pp 125 and 131.