Synthesis of Some Benzofuran and Furocoumarin Derivatives for Possible Biological Activity

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Received December 18, 1980, *from the National Research Centre, Dokki, Cairo, Egypt.* Accepted for publication October 15, 1981. *Present address, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt.

Abstract □ Condensation of 5-formyl-6-methoxy-2,3-diphenylbenzofuran (I) and 6-formyl-5-methoxy-2,3-diphenylbenzofuran (II) with aliphatic or aromatic primary amines led to the formation of the corresponding anils (III*a–k* and IV*a–c*). The anils (III*a,f,k* or IV*a–c*) reacted with ethyl cyanoacetate, ethyl acetoacetate, or diethyl malonate to form the respective esters (V*a–c* or VI*a–c*). When V*a–c* or VI*a–c* were treated with pyridine hydrochloride, demethylation occurred followed by cyclization to form the corresponding furocoumarins (VII*a–c* or VIII*a–c*). Reduction of the anils using sodium borohydride furnished the corresponding Mannich bases (X*a–d* and XI). The antimicrobial activity of compounds III*i*, IV*c*, V*a*, VI*a*, and VIII*a* was investigated.

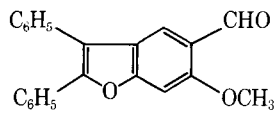
Keyphrases □ Benzofuran—derivatives, synthesis for possible biological activity □ Furocoumarin—derivatives, synthesis for possible biological activity □ Antimicrobial activity—synthesis of some benzofuran and furocoumarin derivatives for possible biological activity

Furocoumarins (1–3) are used as photosensitizing agents, and some of their derivatives possess tuberculostatic (4) as well as molluskicidal (5) activity. Following up a previous investigation (6), synthesis of some new furocoumarins (VII and VIII) derived from 5-formyl-6-methoxy-2,3-diphenylbenzofuran (I) (7) and 6-formyl-5-methoxy-2,3-diphenylbenzofuran (II) (6, 7) was undertaken to investigate these compounds.

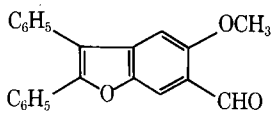
DISCUSSION

Condensation of I or II with equimolar quantities of the appropriate amines gave the corresponding anils (III or IV), respectively. The amines employed were propyl-, isopropyl-, hexyl-, cyclohexyl-, benzylamine; aniline; *m*-toluidine; *p*-toluidine; *p*-anisidine; and *m*-chloro- and *p*-chloroaniline.

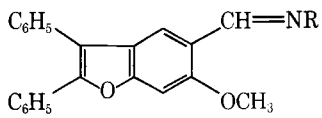
The structure assigned to these anils was supported by IR spectra,



I

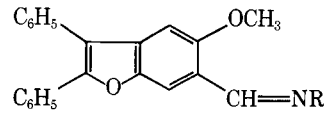


II



III

a, R=$(CH_2)_2CH_3$
b, R=$CH(CH_3)_2$
c, R=$(CH_2)_5CH_3$
d, R=cyclohexyl
e, R=$CH_2C_6H_5$
f, R=C_6H_5
g, R=$C_6H_4CH_3$ (*meta*)
h, R=$C_6H_4CH_3$ (*para*)
i, R=$C_6H_4OCH_3$ (*para*)
j, R=C_6H_4Cl (*meta*)
k, R=C_6H_4Cl (*para*)

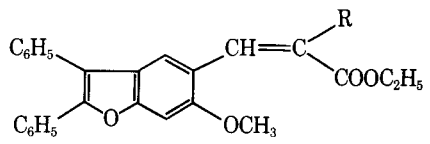


IV

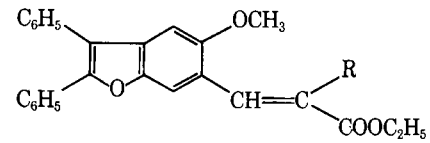
a, R=C_6H_5
b, R=$C_6H_4CH_3$ (*para*)
c, R=$C_6H_4OCH_3$ (*para*)

which showed absorption at 1625–1635 cm^{-1} assignable to —CH=N (8). The PMR spectrum of III*e* showed singlets at δ 3.92 (OCH_3, 3H), 4.84 (CH_2—, 2H), 7.08 (C-4, 1H), and 8.18 (C-7, 1H). The aromatic protons appeared as multiplets at 7.40–7.66 (15H). The mass spectra of III*e* and *k* showed intense molecular ions (M^+) at *m/z* (relative intensity) 417 and 437/439, respectively. In both spectra the imino moieties were eliminated yielding a base peak at *m/z* 311.

Compounds III*e*, *f*, or *k* and IV*a–c* reacted with ethyl cyanoacetate in dry benzene to give the respective esters, V*a* and VI*a*. Compounds V*a* and VI*a* were also obtained by the reaction of I and II with ethyl cyanoacetate in the presence of piperidine. The IR spectrum of V*a* revealed a band at 2220 cm^{-1} characteristic for C≡N group and a band at 1705

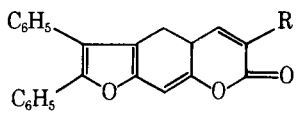


V

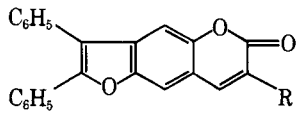


VI

a, R=CN
b, R=$COCH_3$
c, R=$COOC_2H_5$

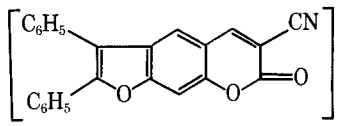


VII

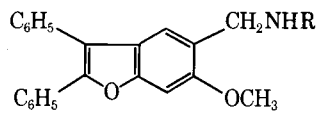


VIII

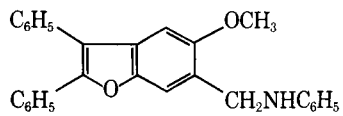
a, R=H
b, R=$COCH_3$
c, R=$COOC_2H_5$



IX



X



XI

a, R=$(CH_2)_2CH_3$
b, R=C_6H_5
c, R=$C_6H_4OCH_3$ (*para*)
d, R=C_6H_4Cl (*para*)

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Table I—Physical Data for Anils IIIa–k and IVa–c

Compound	mp	Yield, %	Formula	Analysis Calc.	Analysis Found
IIIa	142–144	74	$C_{25}H_{23}NO_2$	C 81.31 H 6.23 N 3.79	C 81.10 H 5.96 N 3.52
IIIb	138–140	79	$C_{25}H_{23}NO_2$	C 81.31 H 6.23 N 3.79	C 40.96 H 5.91 N 3.47
IIIc	146–148	67	$C_{28}H_{29}NO_2$	C 81.75 H 7.06 N 3.40	C 81.72 H 6.89 N 3.49
IIId	154–156	78	$C_{28}H_{29}NO_2$	C 82.15 H 6.60 N 3.42	C 82.40 H 6.30 N 3.47
IIIe	163–165	85	$C_{29}H_{23}NO_2$	C 83.45 H 5.52 N 3.36	C 83.60 H 5.55 N 3.25
IIIf	192–194	91	$C_{28}N_{21}NO_2$	C 83.37 H 5.21 N 3.47	C 83.62 H 5.53 N 3.35
IIIg	155–157	74	$C_{29}H_{23}NO_2$	C 83.45 H 5.52 N 3.36	C 83.68 H 5.50 N 3.26
IIIh	180–182	63	$C_{29}H_{23}NO_2$	C 83.45 H 5.52 N 3.36	C 83.32 H 5.61 N 3.01
IIIi	148–150	77	$C_{29}H_{23}NO_3$	C 80.37 H 5.31 N 3.23	C 80.68 H 5.52 N 3.43
IIIj	148–150	86	$C_{28}H_{20}ClNO_2$	C 76.80 H 4.57 N 3.20 Cl 8.12	C 76.82 H 4.83 N 2.84 Cl 7.89
IIIk	162–164	78	$C_{28}H_{20}ClNO_2$	C 76.80 H 4.57 N 3.20 Cl 8.12	C 76.62 H 4.83 N 6.92 Cl 7.79
IVa	128–130	72	$C_{28}H_{21}NO_2$	C 83.37 H 5.21 N 3.47	C 83.44 H 5.46 N 3.24
IVb	150–152	76	$C_{29}H_{23}NO_2$	C 83.45 H 5.52 N 3.36	C 83.51 H 5.77 N 3.09
IVc	163–165	61	$C_{29}H_{23}NO_3$	C 80.37 H 5.31 N 3.23	C 79.99 H 5.32 N 2.94

Table II—Physical Data for Esters Va–c and VIa–c

Compound	mp	Yield, %	Formula	Analysis Calc.	Analysis Found
Va	192–194	74	$C_{27}H_{21}NO_4$	C 76.60 H 4.96 N 3.31	C 76.82 H 5.07 N 3.72
Vb	177–179	71	$C_{28}H_{24}O_5$	C 76.36 H 5.45	C 76.50 H 5.73
Vc	167–169	73	$C_{29}H_{26}O_6$	C 74.04 H 5.53	C 74.46 H 5.74
VIa	192–194	86	$C_{27}H_{21}NO_4$	C 76.60 H 4.96 N 3.31	C 76.45 H 5.23 N 2.99
VIb	168–170	76	$C_{28}H_{24}O_5$	C 76.36 H 5.45	C 76.68 H 5.50
VIc	103–105	75	$C_{29}H_{26}O_6$	C 74.04 H 5.53	C 74.39 H 5.29

cm^{-1}, indicating an ester carbonyl ($—COOC_2H_5$). The mass spectrum of Va showed an M^+ at m/z (relative intensity) 423.

When Va or VIa were treated with pyridine hydrochloride, the furocoumarins VIIa or VIIIa were formed, respectively. The reaction involved demethylation followed by cyclization to form the intermediate derivative IX, which underwent hydrolysis followed by decarboxylation.

The IR spectrum of VIIa showed a band at 1725 cm^{-1} characteristic for the >C=O vibration of coumarins (9). Moreover, absorption bands, corresponding to C≡N and ester >C=O, were not present as in the parent ester Va. The mass spectrum of VIIa showed an M^+ at m/z (relative intensity) 338.

In a similar manner, reaction of IIIf or IVa with ethyl acetoacetate and diethyl malonate yielded Vb and c or VIb and c, respectively.

The mass spectrum of Vb and c showed an M^+ at m/z 440 and 470, respectively.

Treatment of Vb and c and VIb and c with pyridine hydrochloride yielded the corresponding acetyl and carboethoxyfurocoumarin derivatives, VIIb and c and VIIIb and c, respectively.

The structures of VIIb and c and VIIIb and c were confirmed by their melting points and mixed melting points with authentic samples prepared by the reaction of I and II with ethyl acetoacetate and diethyl malonate in the presence of piperidine (7).

Sodium borohydride reduction of the benzofuran anils, IIIa, f, i, and k and IVa, were carried out in ethanol and gave the corresponding Mannich bases Xa–d and XI.

The IR spectrum of Xb showed absorption at 3380 cm^{-1} due to NH

Table III—Physical Data of Mannich Bases X*a–d* and XI

Compound	mp	Yield, %	Formula	Analysis Calc.	Found
X*a*	184–186	69	$C_{25}H_{25}NO_2$	C 80.86 H 6.74 N 3.77	C 81.23 H 7.01 N 3.98
X*b*	154–156	80	$C_{28}H_{23}NO_2$	C 82.96 H 5.68 N 3.46	C 83.17 H 5.82 N 3.58
X*c*	148–150	66	$C_{29}H_{25}NO_3$	C 80.00 H 5.75 N 3.22	C 79.69 H 5.60 N 2.97
X*d*	103–105	53	$C_{28}H_{22}ClNO_2$	C 76.45 H 5.01 Cl 8.08 N 3.19	C 76.71 H 5.35 Cl 8.24 N 3.22
X*I*	124–126	47	$C_{28}H_{23}NO_2$	C 82.96 H 5.68 N 3.45	C 82.76 H 5.80 N 3.07

and no absorption characteristic for C=N appeared. The PMR spectrum of X*b* revealed singlets at δ 3.85 (OCH_3, 3H), 4.40 (CH_2, 2H), 6.75 (C-4, 1H), and 7.72 (C-7, 1H). The NH proton appeared as a broad band at δ 4.00 which disappeared after adding deuterium oxide. Finally, the multiplets at δ 7.17–7.46 corresponded to 15 aromatic protons. The mass spectrum of X*b* showd an M^+ at *m/z* (relative intensity) 405 as a base peak.

EXPERIMENTAL[1]

Studies for Antimicrobial Activity—The activity of some compounds were tested on *Bacillus subtilis*, NRRL 543; *Escherichia coli*, NRRL 210; *Klebsiella pneumoniae*, NRRL 117; *Proteus mirabilis; Sarcina lutea; Salmonella typhosea*, NRRL 537 (bacteria); *Candida lypolitica; Candida pellicula; Sacharomyces cerviceae*, NRRL Y567 (yeasts); *Aspergillus nigar*, NRRL 599; *Fusarium moniliforme*, and *Penicillium funiculosum* (fungi[2]).

Preparation of Benzofuran Anils (III*a–k* and IV*a–c*)—The appropriate amine (0.03 mole) and piperidine (0.5 ml) were added to a solution of I or II (0.03 mole) in benzene or ethanol (50 ml). The reaction mixture was refluxed for 3 hr, then concentrated to ~10 ml and left to cool. The solid was filtered and crystallized from ethanol to give III*a–k* and IV*a–c* as white-yellow crystals (Table I).

Preparation of the Esters (V*a–c* and VI*a–c*)—*Method A*—A mixture of III or IV (1 g) in dry benzene (20 ml) and either ethyl cyanoacetate, ethyl acetoacetate, or diethyl malonate (1.5 ml) was stirred for 0.5 hr and left overnight. The solvent was evaporated under reduced pressure and 5 ml of water was added. The solution was heated and then left to cool. The solid that separated was filtered and crystallized from ethanol to give V*a–c* or VI*a–c* as yellow crystals.

Method B—A mixture of I or II (1 g), 1.5 ml of ethyl cyanoacetate, ethyl acetoacetate, and diethyl malonate esters and piperidine (0.5 ml) in ethanol (50 ml) was refluxed for 1 hr and left to cool. The solid that formed was crystallized from ethanol as yellow crystals.

Methods A and B gave the same products (melting point and mixed melting point gave no depression) (Table II).

Action of Pyridine Hydrochloride on V*a–c* and VI*a–c*—A mixture of V*a–c* or VI*a–c* (1 g) and freshly prepared pyridine hydrochloride (3 g) was kept at 200° for 20 min, left to cool, then acidified with dilute hydrochloric acid. The solid was filtered and crystallized from an appropriate solvent.

Compound VII*a* was obtained at a 56% yield as pale brownish crystals from ethanol, mp 223–225°.

Anal.—Calc. for $C_{23}H_{14}O_3$: C, 81.66; H, 4.14. Found: C, 81.93; H, 3.99.

Compound VII*b* was crystallized from ethanol as pale brownish crystals, mp 256–258°; yield 55% (melting point and mixed melting point with an authentic sample (7) gave no depression).

Compound VII*c* was obtained as pale brownish crystals from ethanol, mp 196–198°; yield 34% (melting point and mixed melting point with an authentic sample (7) gave no depression).

Compound VIII*a* gave a 53% yield as pale brownish crystals from methanol, mp 220–222°.

Anal.—Calc. for $C_{23}H_{14}O_3$: C, 81.66; H, 4.14. Found: C, 81.41; H, 4.36.

Compound VIII*b* produced a 49% yield as pale brownish crystals from ethanol, mp 256° (melting point and mixed melting point with an authentic sample (7) gave no depression).

Compound VIII*c* gave a 52% yield as pale brownish crystals from acetone, mp 195° (melting point and mixed melting point with an authentic sample (7) gave no depression).

Preparation of the Mannich Bases (X*a–d* and XI)—A mixture of III*c, f, i, k*, or IV*a* (1 g) and sodium borohydride (2 g) in ethanol (50 ml) was heated at 50° for 5 min to start the reaction. The reaction mixture was stirred for 2 hr, and 20 ml water was added and left overnight. The solution was filtered, and the solid washed with water and crystallized from ethanol to give the Mannich bases X*a–d* and XI as colorless needles (Table III).

Antimicrobial Activity—A freshly prepared suspension of the test organisms was used to inoculate three plates, each with nutrient agar (bacterial strain), media containing glucose (10 g), yeast extract (3 g), peptone (5 g), and agar (20 g). All ingredients were dissolved in 1 liter of distilled water and adjusted at pH 6.0. The fungi were cultivated on Dox's agar plates. Fungal inoculum was prepared from 14-day old cultures. The paper disk method was used to test III*i*, IV*c*, V*a*, VI*a*, and VII*a*; one representing each series selected at random to screen their antimicrobial activity. A filter paper disk (5-mm diameter) containing 100 μg from each compound was soaked, dried, and firmly applied to the surface of the inoculated agar plates and then the plates with the bacterial strain were incubated at 37° for 48 hr, while those containing yeast and fungi were

Table IV—Antimicrobial Activity of Compounds III*i*, IV*c*, V*a*, VI*a*, and VII*a* Against Bacteria, Yeasts, and Fungi

Micro-organism	Compound III*i*	IV*c*	V*a*	VI*a*	VII*a*
Bacillus subtilis	7[a]	11	7	7	—
Escherichia coli	7	11	7	7	7
Klebsiella pneumoniae	—	9	—	7	—
Proteus mirabilis	—	11	—	7	7
Salmonella typhosae	7	9	7	7	—
Sarcina lutea	8	11	7	10	—
Candida lypolitica	8	14	8	8	—
Candida pellicula	8	—	7	8	—
Sacharomyces cerviceae	—	9	—	8	—
Aspergillus nigar	—	—	—	—	—
Fusarium moniliforme	—	—	—	—	—
Penicillium funiculosum	7	17	8	—	—

[a] Diameters of inhibition zone in millimeters.

[1] All melting points are not corrected. The IR spectra were recorded on Carl-Zeiss Spectrophotometer model UR 10. The PMR spectra were run in $CDCl_3$ at 60 MHz, with tetramethylsilane as internal standard on a Varian instrument. Mass spectra were carried out at 70 eV on a Varian Mat 112 Spectrometer.

[2] The organisms with no identification numbers were isolated locally and were isolated by M. M. Fahim, Y. A. Abduo, and M. M. Atalla, Third Egypt. Phytopathol. Congress, Cairo, pp 734–744 (1979).

incubated at 30° for 48–72 hr. The inhibition zone was measured around each disk (Table IV).

CONCLUSIONS

The present results indicate that the microorganisms tested were sensitive to the action of the anils (IIIi and IVc) and the esters (Va and VIa) except for *A. nigar* and *F. moniliforme*. The results also illustrated that the anils potentiate the activity more than the esters. However, it was observed that the presence of the methoxy group in the *para* position to the benzofuran oxygen (IVc and VIa) increased the activity. The cyclization of Va blocked the activity against most of the microorganisms tested. Such preliminary results would encourage further studies to elucidate the relationship between structure and activity.

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Aspirin—A National Survey V: Determination of Aspirin and Impurities in Enteric Coated Tablets and Suppository Formulations and *In Vitro* Dissolution of Enteric Coated Tablets

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Abstract □ The results of a national survey on the quality of enteric coated aspirin tablets and aspirin suppositories are presented. The tablets were analyzed for strength, salicylic acid content, *in vitro* dissolution rate, and related aspirin impurities. The suppositories were analyzed for strength and salicylic acid content. The methods of analysis and validation of data are also presented.

Keyphrases □ Aspirin—semiautomated procedure for enteric coated tablets □ Dissolution—automated *in vitro* profiles of enteric coated aspirin tablets □ Analgesics—determination of aspirin and impurities in enteric coated tablets

A national survey of aspirin tablet products was conducted at the National Center for Drug Analysis in 1978 and 1979 to ascertain the quality of these products.

Parts I–III (1–3) of this series deal with the analysis of aspirin, salicylic acid, and aspirin related impurities in plain and buffered tablets. Part IV (4) compares *in vitro* dissolution results for these dosage forms using both the USP XX paddle and basket procedures (5). The present report describes the quality of enteric coated tablets with respect to content uniformity, dissolution characteristics, and impurities. Suppository formulations were also checked for content uniformity and impurities.

The official compendia do not provide a method or criterion for the *in vitro* dissolution of enteric coated tablets. Embil and Torosian (6) described the dissolution behavior of two brands of enteric coated tablets using a basket procedure. Over 60% of the aspirin content in the two brands dissolved within 3 hr, but there were significant differences in the release rates. Johansen (7) investigated the correlation between dissolution and absorption rates for plain and enteric coated aspirin tablets. The dissolution rate determinations were made with both a Sartorius apparatus and a USP XIX basket apparatus. Johansen found that the USP XIX basket apparatus, when applied to enteric coated tablets, gave a poor *in vitro/in vivo* correlation. He attributed this to the fact that the USP apparatus dissolved aspirin rather quickly after changing from simulated gastric fluid to intestinal fluid. To obtain a better correlation he recommended decreasing the rotational speed of the basket.

The purpose of this study was to investigate a semiau-

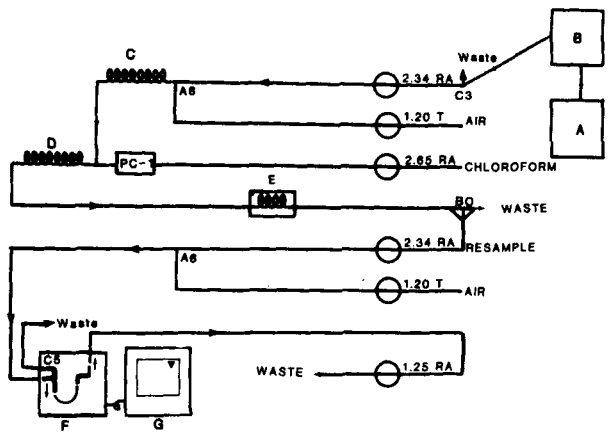

Figure 1—*Flow diagram of automated system for enteric coated aspirin dissolution. Key: (T) Tygon pump tube; (RA) red acidiflex pump tube; (C) 28-turn × 2.4-mm i.d. mixing coil; (D) 28-turn × 2.4-mm i.d. mixing coil with one double end; (E) 5.5-turn setting coil; (F) UV spectrophotometer; (G) recorder; (A) six-spindle dissolution apparatus; (B) automatic sampler. Pump tube sizes are in milliliters per minute. C-3, C-5, A6, and PC-1 are commercially available glass fittings.*

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