Reaction of 3-Benzylidenephthalide with Diamines and p-Aminophenylcarboxylic Acids & Antimicrobial Activities of the Products

A.H.BEDAIR*, R.Q.LAMPHON AND S.A.GHAZAL**

*Department of Natural Sciences, Faculty of Education, King Abdul-Aziz University Medinah Munawwarah, Saudi Arabia.

**Botany Department, Faculty of Science, Al-Azhar University, Nasr-City, Cairo, Egypt.

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Summary: Bis-(2-methylene-3-benzalphthalimidine) II, desoxybenzoin-o-(4'-aminophenyl) carboxamide III and 2-(substitutedphenyl)-3-benzalphthalimidines of type IV and V were obtained via interaction of corresponding diamines with 3-benzylidenephthalide I. Condensation of $C_6H_4(NH_2)_2$-1,2 with I gave fused benzimidazoisoindol derivative VII as anomalous product.

Benzalphthalimidines with p-carboxylate of type VIIIa-c are also prepared. Their IR, PMR and Mass spectra were also studied, III and VIIIb were found to exhibit activity against Gram-positive, Gram-negative, yeast and filamentous fungi.

Although many reactions of 3-benzylidenephthalide I [1] with several nucleophilic reagents have been investigated [2-8], but reactions of I with ethylenediamine, phenylenediamines and p-aminophenylcarboxylic

*Present Address: Chemistry Dept. Faculty of Science Al-Azhar University, Nasr-City, Cairo, Egypt.

**Botany Department, Faculty of Science, Al-Azhar University, Nasr-City, Cairo, Egypt.

acids have not been studied. In the present investigation, action of these reagents on I have been studied and their antimicrobial activities [9] were also discussed.

Condensation of I with ethylenediamine under mild conditions furnished only one product II. Elemental analysis of this product showed a value compatible with molecular formula $C_{32}H_{24}O_2N_2$ which indicates that one mole of ethylenediamine reacted with two moles of I. The IR spectrum of this product showed νC=O as a broad band at 1670 cm^{-1} and νC-H aliphatic at (2920 - 2840 cm^{-1}) respectively. The mass spectrum showed a molecular ion peak at m/z 468 (M^+) together with a base peak at m/z 248 and other peaks at m/z 395, 394, 377, 288, 232 and 221. The above findings led to the formulation of this product as Bis-(2-methylene-3-benzalphthalimidine) II. However, condensation of I with p-phenylenediamine in glacial acetic acid containing fused sodium acetate furnished two products. Elemental analysis of these products showed values compatible with molecular formulae $C_{21}H_{18}N_2O_2$ and $C_{23}H_{18}N_2O_2$.

The IR spectrum of one of the products ($C_{21}H_{18}N_2O_2$) showed absorption bands at 3425 (νNH_2), 3337 (,NHCO) and at 1675 cm^{-1} (νC=O). The mass spectrum showed a molecular ion peak at m/z 330 (M^+) together with a base peak at m/z 312.

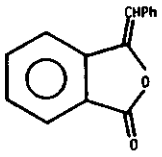
The above findings led to the formulation of this product as desoxybenzoin-o-(4'-aminophenyl) carboxamide III.

The second product $C_{23}H_{18}N_2O_2$ was assigned the structure 2-(p-acetaminophenyl)-3-benzalphthalimidine IV on the basis of spectral data. Its IR spectrum showed absorption bands at 3330 (νNH) and at 1690 cm^{-1} (νC=O). The PMR spectrum of this compound exhibited signals at δ 2.1 (3H, s, $COCH_3$), 6.48 [1H, s, (-C=CH-)] and 7.1 - 8.3 [14H, m, Ar - H & NH] ppm. The mass spectrum showed a molecular ion peak at m/z 354 (M^+) (100 %) as a base peak together with other peaks at 312, 311, 296, 295, 277, 235, 192, 150, 108, 106 and 91.

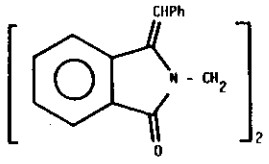
It is clear that 3-benzylidenephthalide I must have opened with p-phenylenediamine to give III which underwent cyclization with subsequent acetylation of the free amino group to phthalimidine compound IV.

Interaction of I with o-nitro-p-phenylenediamine furnished the corresponding 2-(o-nitro-p-aminophenyl) 3-benzalphthalimidine V. The structure was supported by analysis, IR spectrum showed νCO at 1710 as strong absorption band, νNH at 3340, 3490 and νNO_2 at 1375 and 1520 cm^{-1}. The PMR spectrum of this compound showed five sets of signals at δ 6.28 [2H, s, NH_2 (exchanged with D_2O)], 6.33 [1H, d, Hd (which showed long range spin coupling with H^*)(JHdH* = 2.68Hz)] [10], 6.93 (1H, d, Hb; JHbc = 8.57Hz), 8.12 [2H, confused signal, (Ha & H^*)] and at 7.20 - 7.82 (9H, m, Ar-H) ppm. The proton Hc appeared as a doublet at δ = 7.85 ppm, after shaking with D_2O. The mass spectrum shows molecular ion peak at m/z 357 (100%) as base peak together with other peaks at 339, 323, 309, 294, 214, 77, 75 and 73.

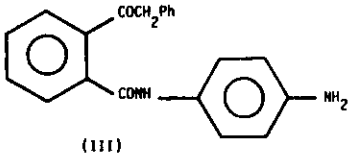
On the contrary the interaction of I with o-phenylenediamine in glacial acetic acid in the presence of fused sodium acetate furnished one product only. Elemental analysis and both its



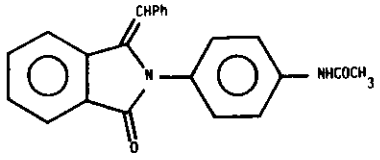
(I)



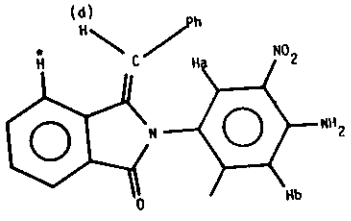
(II)



(III)



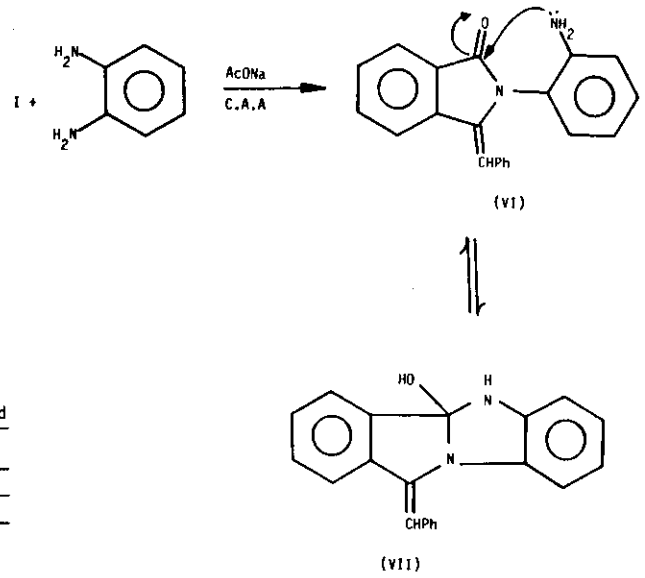
(IV)



(V)

IR and PMR spectra confirmed that this product may be assigned structure 6-(benzal)benzimidazo[2,3-c] isoindo-10a - ol **VII** rather that the expected structure **VI** which could not be isolated. The IR spectrum of **VII** shows the disappearance of $\nu C=O$, while PMR spectrum showed two absorption signals at δ 4.55 and 7.2 - 8.2 in the intensity ratio 1:15. The first signal at δ4.55 [1H, broad signal (exchangeable with D_2O), OH] and the second signal atδ7.2 - 8.2 [15H, m, (Ar - H, -C = CH -, NH)] ppm.

The formation of product **VII** was rationalized through the following sequence:



Scheme-1

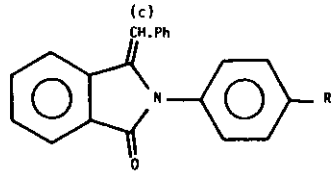
In a similar fashion, condensation of I with ethyl p-aminobenzoate, p-aminophenylacetic acid and with p-aminohippuric acid was successful and the corresponding benzylidene-phthalimidines **VIIIa-c** with p-carboxy-late group were obtained.

The assignment of structure **VIII** was based on analysis, IR measure-ments which showed $\nu C=O$ at 1710, 1760 cm^{-1}, $\nu C-H$ (aliphatic) at (2930

Table-1: Characterization Data of Various Compounds prepared

Compds	M.P. °C	Formula	Analysis Found / Calc. C%	H%	N%
II	240-242	$C_{32}H_{24}N_2O_2$	82.00 82.05	5.20 5.13	5.88 5.98
III	220	$C_{21}H_{18}N_2O_2$	76.12 76.36	5.32 5.45	8.54 8.48
IV	260-262 (dec.)	$C_{23}H_{18}N_2O_2$	77.99 77.97	5.10 5.08	7.90 7.91
V	192-194	$C_{21}H_{15}N_3O_3$	70.46 70.59	4.32 4.20	11.76 11.76
VII	158-160	$C_{21}H_{16}N_2O$	80.49 80.77	5.90 5.13	8.88 8.97
VIIIa	96-98	$C_{24}H_{19}NO_3$	78.10 78.05	5.38 5.15	3.80 3.79
VIIIb	204-206	$C_{23}H_{17}NO_3$	77.55 77.75	4.99 4.79	3.86 3.94
VIIIc	230	$C_{24}H_{18}N_2O_4$	72.50 72.36	4.40 4.52	7.10 7.04

- compound VIIIa crystallised from petroleum ether (60-80°).
- compounds VIIIb,c crystallised from ethanol.



(VIII)

(b) (a)

VIIIa ; R = $COOCH_2CH_3$

b ; R = CH_2COOH

c ; R = $-C(=O)-N(H)-CH_2-COOH \rightleftharpoons C(OH)=N-CH_2-COOH$ (A) (B)

- 2850 cm^{-1}) (compound **VIIIa**); νC=O at 1680 cm^{-1} and νCOOH at (3300 - 2750 cm^{-1}) (compound **VIIIb**); νC=O at (1740 - 1690 cm^{-1}), νCOOH at (3380 - 2780 cm^{-1}) and NH at 3410 cm^{-1} (compound **VIIIc**). PMR spectrum of compound **VIIIa** showed four signals in the intensity ratio 3:2:1:13 at : δ 1.37 [3H, t, CH_3, J = 6Hz], 4.33 [2H, q, CH_2, J = 6Hz], 6.35 [1H, s, -CH=C] and at δ 7.25 - 8.00 (13H, m, Ar-H) ppm. Whereas PMR spectrum of compound **VIIIc** (DMSO) showed four signals at δ 3.95 [2H, d, CH_2; J_{gem} = 8Hz], 5.95 (1H, br, OH), 8.75 (1H, s, NH) (these two signals were exchanged by D_2O) and at 7.4 - 8 [14H, m, Ar-H and -CH = C-(c)] ppm; it is clear that compound **VIIIc** was present in tautomeric forms A and B. The intensity of the upper-field singlet at δ 5.95 is much greater than that of the downfield singlet at δ 8.75 which indicates that the tautomeric form B is the most predominant one.

The mass spectrum of compound **VIIIc** give rise to the molecular ion peak at m/z 398 (M^+) together with base peak at 250 and other peaks at 380, 352, 324, 306, 280, 267, 222, 204, 194, 104, 76.

Antimicrobial Activity:

Antimicrobial testing of the above mentioned compounds was carried out and it was found (Table-2) that compound **VIIIb** showed activity against a number of Gram-positive bacteria; Micrococcus luteus (maximum activity) (++++) with MIC = 7.5 μg/ml, Bacillus subtilis (moderate activity) (+++) with MIC = 10μg/ml, Bacillus cereus (slight activity) (++) with MIC = 12.5μg/ml and against Gram-negative bacteria, Escherichia coli (slight activity (++) with MIC = 12.5μg/ml. Also MIC 12.5 μg/ml against unicellular yeast, Candida utilis and filamentous fungi, Trichophyton sp. while other compounds were inactive. Thus, it is obvious that the presence of free functional group ($-CH_2COOH$) at the 4'-position of 2-phenylphthalimidine part (compound **VIIIb**) causes a pronounced effect on the antimicrobial activity.

Experimental

The melting points are uncorrected, the IR spectra (Nujol) were measured on Perkin Elmer spectrophotometer, PMR spectra ($CDCl_3$) were measured on Varian PM 390 90MHz NMR spectrophotometer and Mass spectra on Micromass 168 V.G. Micromass Ltd. Analytical data were determined in the microanalaytical unit, Leicester University, U.K.

Bis(2-methylene-3-benzalphthalimidine) II (Table 1):

A solution of I (4.44g; 0.02 mol) in abs. ethanol (20ml) was treated with ethylenediamine (0.02 mol) dropwise with occasional shaking. The reaction mixture was left aside up to 30°C overnight, the solid obtained was filtered off and recrystallised from ethanol to give **II** as colourless crystals (yield 26%).

Table-2: Antimicrobial Activity
(++++ = maximum activity, MIC* 7.50 ; +++ = moderate activity, MIC 10 ; ++ = slight activity, MIC 12.5 and - = inactive).

Type of Organisms		II	III	IV	V	VII	VIIIa	VIIIb	VIIIc
Gram-positive	Micrococcus luteus	-	-	-	-	-	-	++++	-
	Bacillus subtilis	-	-	-	-	-	-	+++	-
	Bacillus cereus	-	-	-	-	-	-	++	-
Gram-negative	Escherichia coli	-	-	-	-	-	-	++	-
Yeast	Candida utilis	-	++	-	-	-	-	++	-
Fungi	Trichophyton sp.	-	++	-	-	-	-	++	-

* Minimal inhibitory concentration (MIC in µg/ml).

Condensation of I with phenylenediamines : General Procedure:

A mixture of **I** (0.05 mol) and phenylenediamine compound (0.05 mol) in glacial acetic acid (30ml) containing (0.2g) freshly fused sodium acetate were heated under reflux for 6 hour, cooled and decomposed with dil. HCl.

a. In the case of p-phenylenediamine, the product obtained was treated with ethanol in which the yellow solid dissolved yielding **III** (28%). The insoluble residue on crystallisation from xylene gave IV (26%) as light brown crystals (Table 1).

b. In the case of o-nitro-p-phenylenediamine, the product was filtered and recrystallised from ethanol to give **V** as golden yellow needles (yield 52%) (Table 1).

c. In the case of o-phenylenediamine, the product was filtered and recrystallised from dil. ethanol to give **VII** as yellow needles (yield 40%) (Table 1).

Benzylidenephthalimidine-p-carboxylate (VIIIa-c) (Table 1):

The method was proceeded as above in the case of **III**, **IV**, **V** and **VII**.

Biological Screening (Table 2):

The compounds were tested against different types of Gram-positive bacteria (Micrococcus luteus, Bacillus subtilis, Bacillus cereus), Gram-negative bacteria (Escherichia coli), unicellular yeast (Candida utilis) and filamentous fungi (Trichophyton sp.) using the hole plate and filter paper disc method [11-13].

A quantitative assays were done on active compounds only.

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