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AFLATOXIN ANALOGUES AS POSSIBLE ANTICOAGULANTS—I

G. V. P. CHANDRA MOULI,* Y. D. REDDY and V. V. SOMAYAJULU

Department of Chemistry, Regional Engineering College, Warangal-506 004, India

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Abstract—3-Substituted-7a, 10a-dihydro-4-hydroxy-5-methoxy-2H-furo(3',2';4,5)furo(2,3-*h*) benzopyran-2,9(9H)-diones and related compounds which contain the carbon skeleton of aflatoxins, were synthesized. They exhibited medium anticoagulant activity at early stages.

Aflatoxins are a group of mycotoxins which are highly oxygenated and unsaturated compounds. They have been gaining increasing significance as food contaminants[1] and carcinogens.[2] Aflatoxin-B_1 is recognised as the most active hepato carcinogen yet discovered.[3] The seriousness of aflatoxicosis in the realms of health, nutrition and agriculture, coupled with the discovery of new naturally occuring carcinogens, prompted reports from different laboratories.[4]* The contributions of Buchi *et al.*[5] and Roberts *et al.*[6] are outstanding. A striking characteristic of all these compounds is the presence of a structural feature which has not been previously noticed in the field of natural products. This feature is the presence of di-(or tetra)hydrofuro-(2,3-*b*)benzofuran[7] fused to a 2-oxo-pyran unit.[8]

Our initial interest in this field was prompted by the stress given to the presence of 4-hydroxy-2H benzopyran-2-one unit in many of the widely used anticoagulants.[9] We have undertaken an investigation of the synthesis of 3-substituted-4-hydroxy-2H benzopyran - 2 - ones which contain a tetrahydro furo(2,3-*b*)benzofuran moiety in their molecules, which are analogous to aflatoxins in structure, as a part of an on going programme to study their anticoagulant activity.

RESULTS AND DISCUSSION

2,3,3a,8a - Tetrahydro - 4 - hydroxy - 6 - methoxy - furo - (2,3-*b*)benzofuran (**1**) was prepared starting from phloro-glucinol by the method of Buchi *et al.*[10] Compound (**1**) was allowed to react with substituted malonic acids by the process of Shah *et al.*[11] to give 3 - alkyl - 7a,10a - dihydro - 4 - hydroxy - 5 - methoxy - 2H - furo - (3',2';4,5)furo(2,3-*h*)benzopyran - 2,9(9H) - diones (compounds **2a** and **2b** in Scheme 1).

When formaldehyde,[12] aromatic and heteraryl aldehydes were allowed to react with compound 2a a number of 2,2'-(methylene or benzylidene or heteraryl-methylene-bis[7a,10a-dihydro - 4 - hydroxy - 5 - methoxy - 2H - furo - (3',2';4,5)benzopyran - 2,9(9H)diones (**3a–h**; Scheme 2) have been obtained.

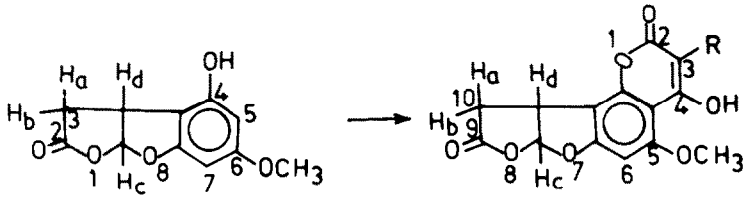
But when the above condensation was carried out with *o*-hydroxy phenyl and naphthyl aldehydes,[13] two different products were isolated depending upon the ratio of reactants from the reaction mixture. If the molar ratio is 1 : 1 the main products were 3 - benzylidene - 10,10a - dihydro - 5 - methoxy - 2H - furo - (3',2';4,5) - furo - (2,3-*h*) - benzopyran - 2,4,9 - (3H, 7aH)triones (**4a–c**; Scheme 3).

If the ratio of compound **2a** to aldehyde is 2 : 1, compounds (**4a–c**) along with a series of 3a,14a - dihydro - 12methoxy - 6 - [7a,9a,10,10a,-tetrahydro - 4 - hydroxy - 5 - methoxy - 2,9 - dioxo - 2H - furo(3',2';4,5)furo(2,3-*h*)benzopyran - 3 - yl]-5H,6H - benzopyrano(3,2-*c*)furo(3',2';4,5)furo(2,3-*h*)benzopyran - 2,5(3H) - diones (**5a–c**; Scheme 3). The mixture was separated by treating it with ethanol. The products **4a–c** are soluble whereas compounds **5a–c** are insoluble in alcohol.

The course of reaction can be indicated by an initial aldol condensation of the aldehyde, dehydration of which leads to the formation of compounds **4a–c**., followed by a Michael reaction between compound (**10**) and a second molecule of **2a**, which eliminates a molecule of water to convert into compounds **5a–c** involving one of the enolic and phenolic hydroxy groups.

In view of the specific physiological activity associated with the 4-hydroxy function of these above compounds, the compounds (**3e**, **3f** and **3h**) have been hydrated by acetic anhydride and pyridine[14] to give 6 - aryl/heteraryl - 3a - 8c,11a,18a - tetrahydro - 14,16 - dimethoxy - 6 - phenyl(1H, 6H, 7H)bisfuro(3',2';4,5)furo(2,3-*h*; 2',3'-*h'*)pyrano(3,2-*c*; 5,6-*c'*)bis benzopyran - 2,5,7,10 - (3H, 9H) - tetraones (**6a–c**; Scheme 5).

†Dedicated to (late) Prof. S. Rajagopal.



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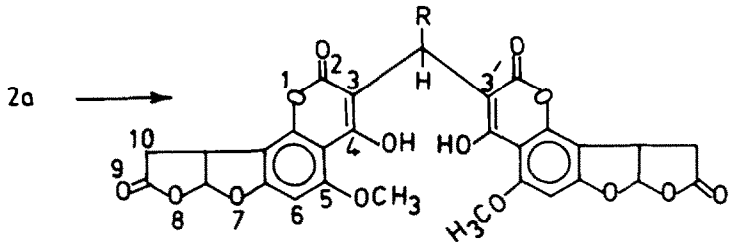
2a, R=-H
2b, R=-CH_3

Scheme 1.



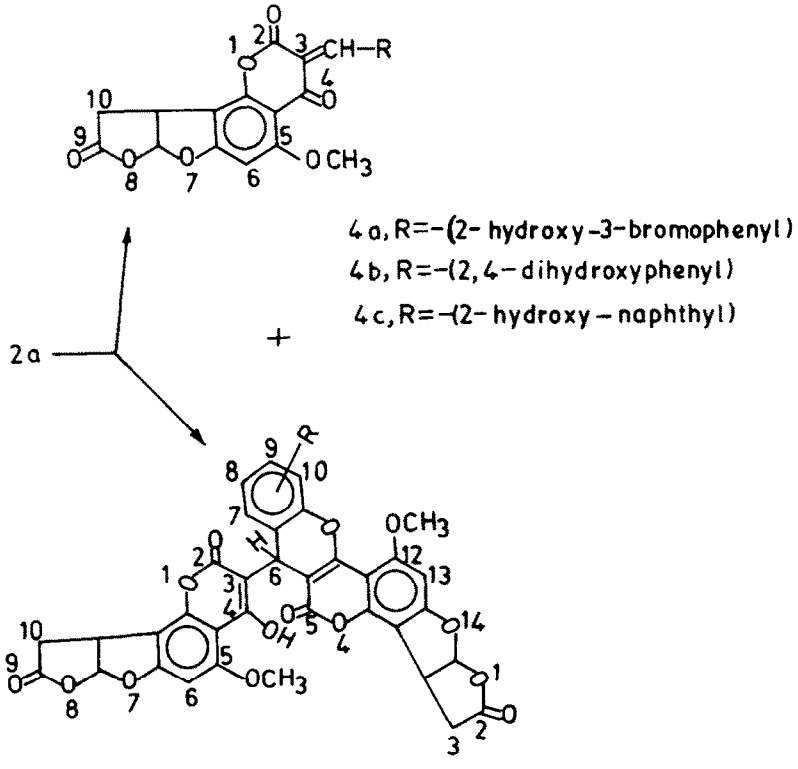
3a, R = −H ; 3b, R = phenyl ; 3c, R = − o − chlorophenyl
3d, R = − p − chlorophenyl ; 3e, R = − p − N, N − dimethyl aminophenyl ; 3f, R = 7 − methoxy − 2H − (1) − benzopyran − 2 − one − 4yl ; 3g, R = 5, 7 − dimethoxy − 2H − (1) − benzopyran − 2 − one − 4yl ; 3h, R = 4H − (1) − benzopyran − 4 − one − 3yl

Scheme 2.

To reduce the number of stages in the synthesis of compound **2a**, an alternative scheme has been designed and attempted. 7 - Methoxy - 5 - hydroxy - 4 - methyl - 2H - benzopyran - 2 - one(7)[15] was condensed with simple malonic acid by Shah's route to give the 4 - methyl - 8 - hydroxy - 9 - methoxy - 2H,6H - benzodipyran (1,2,-*b*; 3,4-*b*) - 2,6 - dione(**8**). The above compound was subjected to oxidation with selenium dioxide to give the corresponding 4 - formyl - 8 - hydroxy - 9 - methoxy - 2H,6H - benzo - dipyran(1,2-*b*; 3,4-*b*)2,6 - dione (**9**).

The4-formyl upon treatment with zinc in acetic acid underwent β-acyl lactone rearrangement[16] to form into compound 2a (Scheme 6). It was observed that in the stages of oxidation and β-acyl lactone rearrangement the yields were not encouraging.

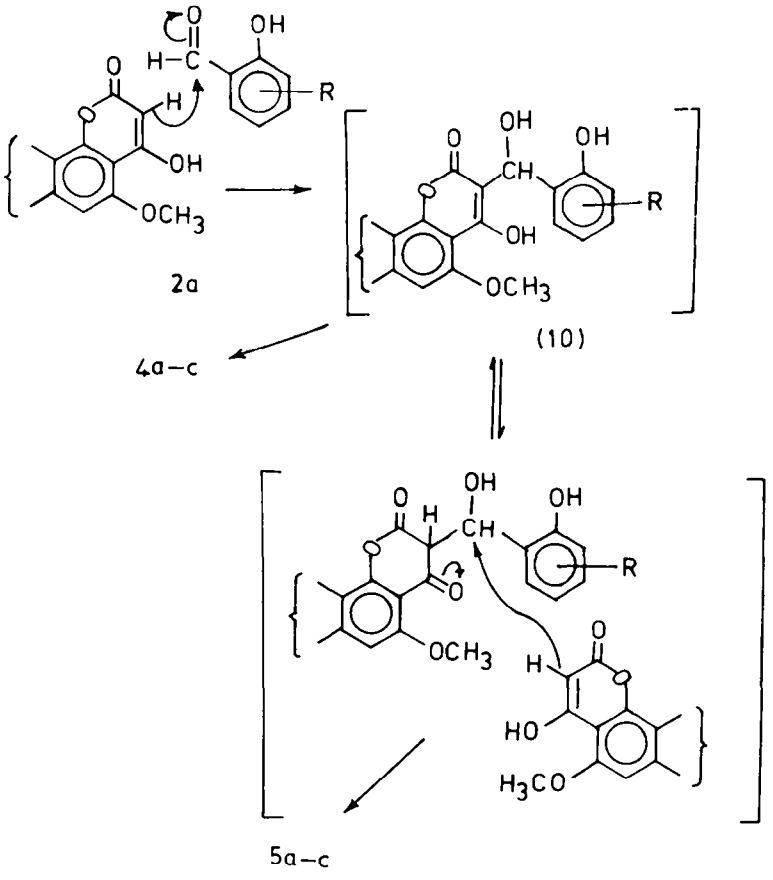
All the structures have been confirmed on the basis of elemental analysis, UV, IR and ^{1}H NMR data. These compounds exhibit general UV absorptions bands in the regions (nm) 225, 275 and 310 (cinnamoyl chromophore) which are similar to the spectra of aflatoxins.[17] The bands are slightly shifted in basic medium, they are



4a, R = −(2 − hydroxy − 3 − bromophenyl)
4b, R = −(2, 4 − dihydroxyphenyl)
4c, R = −(2 − hydroxy − naphthyl)

5a, R = −(10 − bromo)
5b, R = −(9 − hydroxy)
5c, R = −(7, 8 − benzo)

Scheme 3.



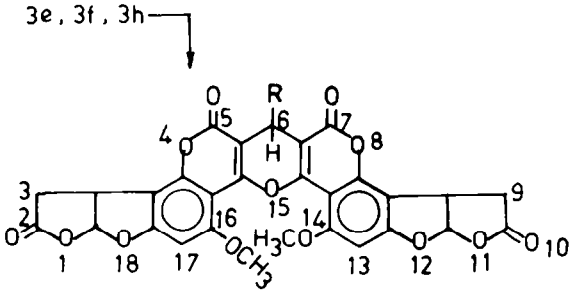
Scheme 4.

located around 217, 242(s), 275(s), 280(m), 302(s)[18] whereas compounds **4a–c** exhibit an additional band in the region 320 nm. The compounds **5a–c** show UV absorption mainly at 230, 280 and 310, 340 nm.

The IR spectra include absorption around 1785, 1760, 1688 cm^{-1}, indicating cis fusion of the five membered system and the presence of γ-lactone and δ-lactone system[19] in all the compounds. The peak at 1785 cm^{-1} is due to γ-lactone anticipated to be an exceptionally good hydrogen bond acceptor.[20]

In the spectra of compounds **2a–b**, **3a–h** and **5a–c**, the enolic hydroxyl is located at 3050 cm^{-1}, and is very broad. In addition, absorptions at 1210, 1080 cm^{-1} for compounds **5a–c** indicate the epoxy linkage. The IR spectra of compounds for **4a–c** are slightly different. The broad band appearing at 1630 cm^{-1} is more intense than the normal carbonyl and is indicative of β-diketone function.[21] The phenolic hydroxyl is observed at 3560–3520 cm^{-1} with no anolic absorption. The aromatic bands are observed in 820, 750, 650 cm^{-1}.

The ^1H NMR spectrum of **2a** contain signals for Ha and Hb (Scheme 1). At δ 2.9–3.2 (2H, d), Hc around δ 5.6–6.0 (1H, d), Hd at δ 3.8–4.0) (1H, m). The aromatic protons is located in the region of δ 6.4–6.9 (1H, s). The

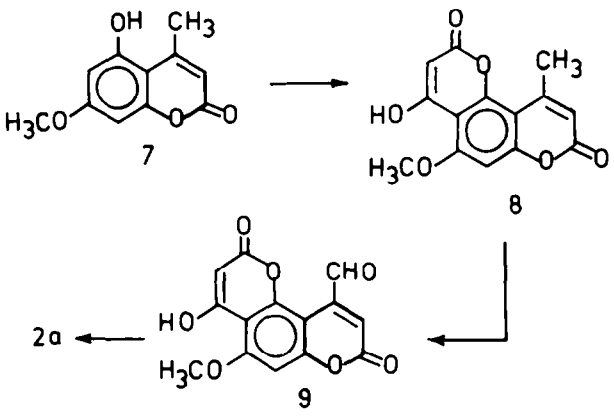


6a, R=N,N-dimethyl amino phenyl.

6b, R=7-methoxy-2H-(1)-benzopyran-2-one-4yl.

6c, R=4H-(1)-benzopyran-4-one-3yl.

Scheme 5.



Scheme 6.

Table 1.

Physical data of the compounds synthesised

Compound	R	Yield %	m.p. (°C)	Mol. formulae.	Found %C	Calc %H	
2a	H	28	248	$C_{14}H_{10}O_7$	57.86 (57.93)	3.51 (3.44)	
2B	CH_3	35	268	$C_{15}H_{12}O_7$	59.27 (59.21)	3.91 (3.94)	
3A	H	21	297	$C_{29}H_{20}O_{14}$	58.92 (58.78)	3.21 (3.37)	
3B	Phenyl	28	314	$C_{35}H_{24}O_{14}$	62.73 (62.87)	3.67 (3.59)	
3c	O-Chlorophenyl	20	318	$C_{35}H_{23}O_{14}Cl$	59.32 (59.78)	3.47 (3.27)	
3d	p-Chlorophenyl	40	302	$C_{35}H_{23}O_{14}Cl$	59.94 (59.78)	3.43 (3.27)	N
3e	p-N,N-Dimethylamino-	27	> 330	$C_{37}H_{29}O_{14}N$	62.59 (62.47)	4.12 (4.08)	1.97 (1.97)
3f	7-Methoxy-2-oxo-2H-benzopyran-4-yl	43	304	$C_{39}H_{26}O_{17}$	61.06 (61.09)	3.48 (3.39)	
3G	4,7-Dimethoxy-2-oxo-2H-benzopyran-4-yl	26	324	$C_{40}H_{28}O_{18}$	60.13 (60.30)	3.42 (3.57)	
3h	4-oxo-4H benzopyran 3-yl	39	328	$C_{38}H_{24}O_{16}$	61.92 (61.95)	3.17 (3.26)	
4a	2-Hydroxy-3-bromo-phenyl	32	> 330	$C_{21}H_{13}O_8Br$	53.41 (53.27)	2.81 (2.74)	
4b	2,4-Dihydroxyphenyl	25	306	$C_{21}H_{14}O_9$	61.39 (61.46)	3.32 (3.41)	
4C	2-Hydroxynaphthyl	27	> 330	$C_{25}H_{16}O_8$	67.63 (67.56)	3.58 (3.60)	

Compound	R	Yield %	m.p. °C	Mol. Formulae.	Found %C	Calc %H	
5a	10-Bromo	28	> 330	$C_{35}H_{21}O_{14}Br$	56.45 (56.37)	2.92 (2.81)	
5b	9-Hydroxy	25	327	$C_{35}H_{22}O_{15}$	61.47 (61.58)	3.31 (3.22)	
5c	7,8-Benzo	20	> 330	$C_{39}H_{24}O_{14}$	65.29 (65.36)	3.47 (3.35)	N
6a	p-N,N-Dimethylamino-phenyl	32	329	$C_{37}H_{27}O_{13}N$	64.13 (64.07)	3.97 (3.89)	2.08 (2.02)
6b	7-Methoxy-2-oxo-2H-benzopyran-4-yl.	27	> 330	$C_{29}H_{24}O_{16}$	62.93 (62.56)	3.41 (3.20)	
6c	4-Oxo-4H-(1)-benzo-pyran-3-yl	28	> 330	$C_{38}H_{22}O_{15}$	63.32 (63.50)	3.13 (3.06)	

vinyl at position 3 is identified at δ 5.4 (1H, s). The signal at δ 3.5 (3H, s) is due to methoxy group. The enolic proton is observed at δ 12.2 (1H, s). In these systems, minute deviations in signals are observed from the observation of Knight.[22]

The ^{1}H NMR signals for the basic skeleton of almost all the compounds are same as given above. The deviation is for compound **3a**, the vinylic (3 position) is absent. The integration for protons was found to be double, indicative of the formation of bis compound. The exocydic methylene protons[23] at δ 2.2–2.3 (2H, s) also offer ample support to the above fact.

One enolic proton is disappearing from the spectra compounds **5a–c**, in addition, a methine proton highly deshielded to δ 8.3 (1H, s) is noted due to the anisotropy of the aromatic ring system.

The enolic protons are totally absent in the spectra of compounds **4a–c** and **6a–c**. For compounds **4a–c** the exocyclic vinylic proton is identified at δ 6.8 (1H, s). Signal at δ 7.8 (1H, s) reveal the additional phenolic proton. The methyne at the ring junction in compounds **6a–c** is deshielded (δ 6.3; 1H, s).

Anticoagulant activity

Some of the compounds synthesized were screened for anticoagulant activity upon albino (Mus raltus) rats at a dose level of 5 mg. The method adopted is due to Quick *et al.*[24] The average increase in prothrombin time of plasma (12.5%) over normal was noted. From this data the relative anticoagulant index was calculated. This index serves as an approximate basis for the comparison of the activity.

Compound.	Increase in prothrombin time over normal (sec)	Relative anticoagulant index
2a	92.8	41.2
2b	44.16	20.13
3a	64.2	57.07
3d	36.5	29.12
4a	3.03	2.51
5a	24.26	16.81
6a	Nil	Nil
6b	Nil	Nil

The above results indicate that the dicoumarol analogues of aflatoxins show more activity. It is interesting to note that the activity might be alone due to the presence of enolic hydroxyl in position[4], as the compound **4a** and the corresponding epoxy compounds **6a** and **6b** exhibit no activity.

EXPERIMENTAL

The UV spectra were recorded in methanolic solution and methanolic sodium hydroxide solution (λ_{max} in nm with log ε values) using Beckmann DK-2A spectrometer. The IR spectra were taken on Perkin–Elmer model 137 (in KBr, ν_{max} cm^{-1}). Only high intensities bands are reported. The ^{1}H NMR spectra were taken in $CDCl_3$ and in DMSO (D_6) on Varian A-60 instruments; chemical shifts are given in ppm down field from TMS as internal standard. Thin layer chromatography was used routinely for monitoring reactions and separations. Plates coated with Merck Silica gel G were developed with 3–5% methanol in chloroform. The melting points are uncorrected.

Preparation of 3 - (*H or methyl*) - 7a,10a - *dihydro* - 4 - *hydroxy* - 5 - *methoxy* - 2H - *furo*(3',2';4,5)*furo*(2,3-h)*benzopyran* - 2,9(9H) - *diones. General method*

A mixture of **1** (0.05 mole), malonic or methyl malonic acid (0.08 mole) anhydrous $ZnCl_2$ (0.12 mole) and phosphorus oxychloride (0.15 mole) was heated with stirring at 65–70°C for 36 h, cooled and decomposed with ice and allowed to stand for 2 h. The resulting crude product was collected, dissolved in 10% sodium carbonate solution and acidified. At about the neutral point some oily material separated and it was removed. Further acidification of the remaining solution gave the required crude product, separated by TLC and again recrystallised from ethanol. IR (ν_{max} in cm^{-1}, KBr 3150 (–OH), 1780 (γ-lactone), 1640 (δ-lactone), 1180 (C–O–C) and 680 (aromatic).

Preparation of 2,2'(*methylene*|*benzylidene*|*heteraryl* - *methylene*)*bis*[7a,10a - *dihydro* - 4 - *hydroxy* - 5 - *methoxy* - 2H - *furo*(3',2';4,5)*furo*(2,3-h)*benzopyran* - 2,9(9H)-*diones*]. *General procedure*

The compound **2a** (0.03 mole) was suspended in water–ethanol (1:2) and treated with formaldehyde, aromatic and heteraryl aldehydes (0.01 mole) in ethanol drop wise with stirring. The reaction mixture was warmed slightly for about 30 min and allowed to stand at room temperature for about 1 h. The separated solid was filtered, washed with cold water, purified by PTIC on silica gel followed by crystallisation with acetic acid. IR (ν_{max} cm^{-1}, 3300 (–OH), 1785 (γ-lactone), 1620 (δ-lactone), 750 (furan breathings) and 680 (aromatic).

Preparation of 7 - (8/9/10 *substituted*) - 3a, 14a - *dihydro* - 12 - *methoxy* - 6 - [7a,9a,10,10a - *tetrahydro* - 4 - *hydroxy* - 5 - *methoxy* - 2,9 - *dioxo* - 2H - *furo*(3',2';4,5)*furo*(2,3-*h*) - 1 - *benzopyran* - 3 - *yl*[5H,6H - *benzopyran*(3,2-*c*)*furo*(3',2';4,5) *furo*(2,3-*h*)*benzopyran* - 2,5 - (3H) - *diones. General procedure*

The compound **2a** (0.02 mole) was dissolved in hot ethanol (12 ml), *o*-hydroxy aromatic aldehydes were added and the mixture was refluxed for 2½ h. The solid which separated was washed with cold ethanol. The insoluble portion was collected; dried and resolved upon PTIC and crystallised from acetic acid. IR (ν_{max}, in cm^{-1}, KBr, 3480 (–OH), 1775 (ν-lactone), 1640 (δ-lactone), 1080 (C–O–C), 750 (furan), 680 (aromatic).

Preparation of 3 - *substituted benzylidene* - 10,10a, *dihydro* - 5 - *methoxy* - 2H - *furo* - (3',2';4,5) - *furo* - (2,3-h) - 1 - *benzopyran* - 2,4,9 (3H, 7aH) - *triones*

The mother liquor from the above reaction mixture was concentrated and the required crude product separated. It was recrystallised from ethyl acetate. IR (ν_{max} in cm^{-1}, Kbr-1770 (γ-lactone), 1635 (β-diketone), 1050 (C–O–C), 750 (furan), 680 (aromatic).

Preparation of 3a,8c,11a,18a-*tetrahydro* - 14,16 - *dimethoxy* - 6 - (*aryl or heteraryl*) - 5H,6H,7H - *bis furo* - (3',2';4,5) - *furo* - (2,3-*h*; 2',3'-*h'*) - *pyrano*(3,2-*c*; 5,6-*c'*) - *bis* (1) *benzopyran* - 2,5,7,10 (3H, 9H) - *letrone*

The compounds (**3e** or **3f** or **3h**) were dissolved in anhydrous pyridine and an equal volume of acetic anhydride was added. The mixture was allowed to stand at room temperature for about 12–15 h, whereby the crude products separated. They were purified by PTIC and recrystallised from ethanol. IR (ν_{max} in cm^{-1}, KBr, 1780 (γ-lactone), 1660 (δ-lactone), 1090 (C–O–C), 750 (furan), 650 (aromatic).

4 - *Methyl* - 8 - *hydroxy* - 9 - *methoxy* - 2H,6H - *benzo* - (1,2 - *b*; 3,4-*b*) - 2,6 - *dione* (**8**)

A mixture of 5-hydroxy - 7 - methoxy - 4 - methyl - 2H(1) - benzo - pyran - 2 - one (**7**) (0.01 mole), malonic acid (0.01 mole), anhydrous $ZnCl_2$ (0.03 mole) and $POCl_3$ (0.05 mole) was heated with stirring at 60–65° for 36 h, cooled and decomposed with ice. The crude product was extracted with 10% sodium carbonate and acidified. An oily layer that separated but was removed. Further acidification of the remaining solution gave the required product,

which was crystallised from alcohol (0.006 mole; m.p. 221°) $C_{14}H_{10}O_6$ requires: C, 61.3; H, 3.64; Found: C, 61.5; H, 3.59%. UV λ_{max}^{EtOH} 225, 252, 280, 320 (log ϵ 4.3684, 3.6763, 4.003, 3.7218). IR 3100 cm^{-1}, 1720, 1680, 1600, 1420, 1310, 1250, 1020, 820, 650. ^1H NMR ($CDCl_3$), δ 2.7 (3H, s), δ 3.2 (3H, s), δ 5.3 (1H, s), δ 5.6 (1H, s), δ 7.8 (1H, s), δ 11.8 (1H, s).

4 - *Formyl* - 8 - *hydroxy* - 9 - *methoxy* - 2H,6H - *benzo* (1,2-*b*; 3,4-*b'*) - *dipyran* - 2,6 - *dione* (**9**)

To a solution of **8** (0.01 m) in 150 ml of xylene, resublimed selenium dioxide (0.012 mole) was added. The mixture was refluxed for 8 h under a Dean and Stark trap and filtered hot. The yellow crystals collected from the cooled filtrate and the second crop obtained from the concentrated mother liquor by addition of benzene. These two crops are mixed and dissolved in dichloromethane to remove the residual selenium metal. The crude product was recrystallised from benzene (0.0013 mole, m.p. 230°). $C_{14}H_8O_7$ requires C, 58.33; H, 2.77. Found: C, 59.01; H, 2.81%. UV λ_{max}^{EtOH} 228, 260, 280, 325 (log ϵ 4.2573, 3.890, 3.220, 4.5450). IR 3050, 1740, 1720, 1680, 1600, 1460, 1320, 1250, 1080, 780, 650 (KBr) Cm^{-1}. ^{1} NMR (DMSO D_6) δ 2.8 (3H, s), δ 3.5 (3H, s), δ 5.61 (1H, s), δ 5.8 (1H, s), δ10.8 (1H, s), δ 11.2 (1H, s).

Preparation of compound **2a**

To **9** (31.2 mmole) in glacial acetic acid (150 ml) at 100°C (0.125 g atom) of zinc dust was added continuously with vigorous stirring. After keeping at 115–120° for 1½ h, the mixture was added to an equal volume of chloroform, filtered and washed with water. By the concentration of the chloroform layer, the required product was obtained in crude form which can be crystallised from acetic acid (13.6 mmole).

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