Pak. j. sci. ind. res., vol. 33, no. 9, September 1990

SYNTHESIS AND MUTAGENICITY OF SOME DIBENZOSUBERENONE DERIVATIVES

F.A. GAD, A.F. EL-FARARGY, M.EL-MOBAYED AND A.F. HAIKAL

Chemistry Department, National Research Centre, Cairo, Egypt

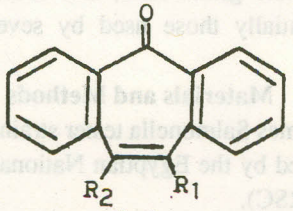
(Received December 28, 1988; revised July 17, 1990)

5-Benzyl, 5-(4-methoxyphenyl) and 5-ethyl or phenyl dibenzosuberen-5-ol derivatives were prepared. Also 10-bromo and 10,11 dibromo dibenzosuberen-5-thioxo were prepared. The given structures were biological screened.

Key words : Mutagenicity, Carcinogenicity, Dibenzosuberenone derivatives.

Introduction

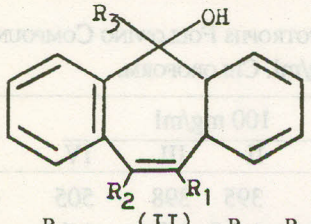
The antidepressant [1-4], antihistaminic [5], anticonvulsive [6] and pharmacological properties [7] of dibenzosuberenone derivatives have made them important chemotherapeutic agents. The present study was undertaken to synthesis some of new dibenzosuberenone derivatives for biological evaluation.



(I)

	R_2	R_1
a	H	H
b	H	Br
c	Br	Br

Thus, dibenzosuberenone [1a), when reacted with benzylmagnesium chloride afforded the 5-benzyldibenzosuberen-5-ol (IIa). 4-Methoxy phenylmagnesium bromide reacted with the same compound and gave 5-(4-methoxyphenyl)-dibenzosuberene-5-ol (IIb). 10-Bromo dibenzosuberenone (Ib) reacted with ethylmagnesium bromide [8] and gave the corresponding 5-ethyldibenzosuberen-5-ol (IIc). Also 10-bromo (Ib) and 10,11-dibromo dibenzosuberenone (Ic)[8] reacted with phenyl magnesium bromide to yield 5-phenyl-10-bromo dibenzosuberen-5-ol (IId) and 5-phenyl 10,11-dibromo dibenzosuberen-5-ol (IIe) respectively.



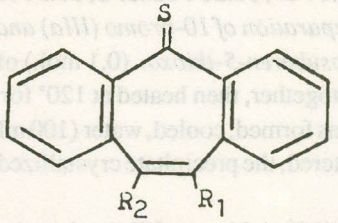
(II)

	R_3	R_2	R_1
a	Ph-CH_2	H	H
b	C_6H_5-OCH_3 (p)	H	H
c	C_2H_5	Br	H
d	Ph	Br	H
e	Ph	Br	Br

Faculty of Science, Zagazig University, Zagazig, Egypt.

The IR-spectrum of (IIc) showed absorption bands at 3060 cm^{-1} (OH), and 2970 cm^{-1} (CH_2), (IIb) showed absorbance at 3060 cm^{-1} (OH), 1605 cm^{-1} (C=C), 2920 cm^{-1} (OCH_3). The NMR-spectrum of (IId) showed a singlet attributed to (CH) at δ 2.8; and the aromatic and hydroxyl protons appeared between δ 6.6-8.2 as a multiplet.

10-Bromo-dibenzosuberenone (1b) and 10,11-dibromo-dibenzosuberenone (Ic)when ground and fused with P_2S_5 at 120° for 10 min, cooled water was added, the dark brown mass formed, left over night, filtered and the precipitate crystallized from methanol in green crystals to give their thioxo derivatives namely, 10-bromodibenzosuberen-5-thioxo (IIIa) and 10,11-dibromodibenzosuberen-5-thioxo (IIIb) respectively.



(III)

	R_1	R_2
a	Br	H
b	Br	Br

The NMR-spectrum of(IIIa) showed a one proton singlet of (CH) at δ 2.8 and multiplet of eight aromatic protons appeared between δ 6.3-8.2.

Experimental

Melting points were recorded in glass capillary tubes and are uncorrected, Microanalysis were performed by the Microanalysis laboratory, National Research Centre, Cairo. IR-spectra were determined using a Carl Zeiss spectrophotometer UR-10. NMR-spectra were obtained in DMSO-d_6 with a varian A 60 spectrophotometer.

Reaction of Grignard reagents with dibenzosuberenone derivatives: General Method. A solution of dibenzosuberenone derivative in THF was added to a solution of alkyl or aryl magnesium halide and the reaction mixture was refluxed for 4 hr., cooled, decomposed with a mixture of crushed ice and dilute hydrochloric acid. The mixture was extracted with ether

and the ethereal extract was evaporated. The residue was crystallized from the proper solvent.

(i) Dibenzosuberenone (Ia) (0.01 mol) reacted with benzyl magnesium chloride (0.03 mol), after 10 hr. of refluxing gave (IIa) (1.6g.; 53.6%), m.p. 59-60° (methanol). $C_{22}H_{18}O$ (298) Calc. C, 88.59 H, 6.04 Found. C, 89.12 H, 5.97.

(ii) (Ia) (0.01 mol) and 4-methoxyphenylmagnesium bromide (0.03 mol), after 4 hr. of refluxing gave (IIb) (1.9 g.; 60.9%), m.p. 82° (methanol). $C_{22}H_{18}O_2$ (314). Calc. C, 84.08 H, 5.73 Found. C, 84.20 H, 5.81.

(iii) 10-bromo dibenzosuberenone (Ib) (0.01 mol.) reacted with ethylmagnesium bromide (0.03 mol.) afforded after 4 hr. of refluxing (IIc) (2.5 gm; 79.4% m.p. 66° (methanol). $C_{17}H_{15}BrO$ (315). Calc. C, 64.76 H, 4.76 Br, 25.40 found. C, 65.10 H, 4.72 Br, 25.28.

(iv) (Ib) (0.01 mol.) reacted with phenylmagnesium bromide (0.03 mol.) and gave after 4 hr. refluxing (IId)(2.1 g.; 73.2%) m.p. 146° (dilute methanol) $C_{21}H_{15}BrO$ (363). Calc. C, 69.42 H, 4.32 Br, 22.04 Found. C, 69.32 H, 4.05 Br, 21.92.

(v) 10,11-dibromo dibenzosuberenone (Ic) (0.01 mol.) reacted with phenylmagensium bromide (0.03 mol.) and gave after 4 hrs refluxing (IIe) (2.9 g.; 62.7%) m.p. 182-3° (methanol + $CHCL_3$) in colourless crystals. $C_{21}H_{14}Br_2O$ (462).Calc. C, 57.04 H, 3.17 Br, 36.20 Found. C, 56.91 H, 3.22 Br, 36.42.

(vi) *Preparation of 10-bromo (IIIa) and 10,11-dibromo (IIIb) dibenzosuberen-5-thioxo.* (0.1 mol.) of (Ib,c) and 5 gm P_2S_5, ground together, then heated at 120° for 10 min., brown black mass was formed, cooled, water (100 ml) was added, left overnight, filtered, the precipitate crystallized from the proper solvent.

(IIIa) in 60% yield, m.p. 97° (methanol + H_2O) $C_{15}H_9BrS$ (301) Calc. Br, 26.24 S, 10.63 Found. Br, 26.80 S, 10.45 (IIIb) in 66% yield, m.p. 155° (cyclohexane) $C_{15}H_8Br_2S$ (380) Calc. Br, 42.11 S, 8.42 Found. Br, 41.91 S, 8.48.

Biological activity. Ames Salmonella strain have been used to screen large numbers of carcinogens for mutagenic activity [9,10]. It was observed that many chemical mutagens are also carcinogenic, and indeed, some compounds were first tested and recogenised as chemical mutagens because they were known to cause tumoure in experimental animals [11]. Several investigators have begun to look at the potency range of mutagenic and carcinogenic properties of carcinogens to determine whether they are similar or dissimilar. If similar, their *in vitro* mutagenicity studies might be useful for estimating the *in vivo* potency of suspect carcinogen [12-14].

These studies have raised hopes that categonies of carcinogenic potency could be determined by *in vitro* mutagenisis assays [15].

Lwoff [16] in his calssical review of lysogency, suggested that prophage introduction in lysogenic bacteria might serve as a test for detecting compounds with mutagenic carcinogenic properties.

Smith [17] reported induced phase lysis in a lysogenic culture of Salmonella thompson by means of nitrogen mustard gas, sulphathiazole glutathione, and sodium thiol acetate. They are essentially those used by several investigators [18-22].

Materials and Methods

Strains. Ames Salmonella tester strains; TA98, TA100, TA1535 supplied by the Egyptian National Research Stock collection (ENRSC).

Schizosaccharomyces pombe mutant ad-D_2 supplied by ENRSC was used in the regulation test.

Media and methods. These are essentially those used by several investigators [18-22].

Results and Discussion

The microbiological studies were performed on four compounds:

(i) 5-Phenyldibenzosuberen-5-ol [7].
(ii) 10-Bromo-5-phenyl dibenzosuberen-5-ol (IId).
(iii) 10-Bromo-dibenzosuberen-5-thioxo (IIIb).
(iv) 10,11-dibromo-dibenzosuberen-5-thioxo (IIIb).

Table 1 represents the mean numbers ± S.D of TA_{98}, TA_{100} and TA_{1535} protorphs following compounds I, II, III and

TABLE 1. AMES TEST SHOWING MEAN NUMBERS ± S.D. OF TA_{98}, TA_{100}, TA_{1535} PROTOTROPHS FOLLOWING COMPOUNDS I, II, III AND IV. TREATMENTS WITH THE DOSES 0,10, 100 AND 200 mg/ml. CHLOROFORM.

Salmonella	O mg/ml				10 mg/ml				100 mg/ml				200 mg/ml		
strains	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III
TA_{98}	12 ± 0.8	345 ± 0.8	345 ± 1.7	345 ± 1.4	–	287 ± 0.8	407 ± 1.4	0 0	79 ± 2.4	395 ± 3.3	398 ± 2.4	505 ± 4.1	475 ± 4.1	–	–
TA_{1535}	50 ± 0.8	0	0	0	–	280 ± 3.3	87 ± 2.4	101 ± 5.3	460 ± 4.9	53 ± 3.7	88 ± 1.6	146 ± 3.3	705 ± 0.8	–	–
TA_{100}	1 ± 0.4	–	–	–	–	–	–	–	4 ± 0.8	–	–	–	9 ± 1.6	–	–

TABLE 2. REGULATION TEST SHOWING THE MEAN NUMBERS ± S.D. OF PINK AND WHITE S. POMBE COLONIES AND TOTAL NUMBERS FOLLOWING COMPOUNDS I,II,III, IV TREATMENTS WITH THE DOSES 0,10, 100 AND 200 mg/1 ml CHLOROFORM.

Salonella strains	O mg/ml				10 mg/ml				100 mg/ml				200 mg/ml			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Pink-colonies x10^3	9 ±0.82	1841.5 ±0.41	1841.5 ± 1.2	1841.5 ± 1.9	–	1 ± 0.47	1 ± 0.41	5.0 ± 0.8	0	30 ± 1.6	4.5 ± 1.2	12.5 ± 2.0	0	–	–	–
White-colonics x10^3	0	1.5 ± 0.08	1.5 ± 0.16	1.5 ± 0.24	–	0	0.5 ± 0.08	0	6 ± 0.8	4.5 ± 1.2	1.5 ± 0.16	3 ± 0.8	2 ± 0.8	–	–	–
Total x 10^3	9	1843	1843	1843	–	1	1.5	5.0	6	34.5	6.0	15.5	2	–	–	–

*Control original suspension differs in different experiments.

IV. Treatments with the doses 0, 10, 100 and 200 mg/ml in chloroform.

From Table 1, it is clear that compound (I) is mutagenic for three Salmonella tester strains as the number of prototrophs increased by increasing the dose, while compound (II) is mutagenic for the strain TA_{98} (frame shift mutant). Its mutagenicity is increased by increasing the dose from 10 mg. The same result i.e. mutagenicity effect appeared in the strain TA_{1535} (base pair substitution mutant) with reduction in the mutagenicity effect by increasing the dose from 10 to 100 mg/ml. Compound (III) is mutagenic in both strains TA_{98} and TA_{1535} and for both doses 10 and 100 mg/ml in chloroform as a solvent. Compound (IV) is highly toxic for the strain TA_{98} for the dose 10 mg/ml while it is highly mutagenic for the strain TA_{1535} in both doses 10 and 100 mg/ml.

Table 2 shows that the compound (I) has lethal effects on S. Pombe cells. It also has positive effects in producing repressed white colonies i.e. it is effective in regulation, while compound (II) shows a great lethality for the dose 10 mg/ml which indicates that this compound is highly toxic at this dose and no change in regulation. The higher dose i.e. 100 mg/ml gave a lesser toxicity than the lower one although larger number of white colonies was obtained which indicate carcinogenicity of this higher dose. Compound (III) showed a high toxicity in both doses 10 and 100 mg/ml, however it is also carcinogenic as the frequency of white colonies was increased. Compound (IV) also is highly toxic in both doses used 10 mg/ml and 100 mg/ml, but the higher dose gave a great toxicity and affected regulation which might indicate carcinogenicity for the higher dose.

Acknowledgement. The author is greatly indebted to Prof. Dr. A.M.M. Ali, microbial genetics Dept., Genetic Engineering, Biotech. Div., National Research Centre for the microbiological activity studies.

References

1. M. J. Haire, (Dupont de Nemours, E.I.); and (O) U.S.4, 256.742 (Cl. 424-244, CO 7D 203, 26); 17 Mars 1981, Appl. 937, 716, 29 Aug. 1978, 13 pp. Cont. in port of U.S. Series No. 937, 716.
2. E. Ciganek, R.T. Uyeda, M.S. Cohen and H. Dewey, J. Med. Chem., **24**, 336 (1981).
3. Engelhardt, L. Edward, (Merck and Co. Inc., U.S. 3922, 305 (Cl. 260-570, 8TC, 207C), 25 Nov. 1975, Appl., 120, 825, 24 May (1961).
4. G.N. Walker (Ciba Geigy Corp.), U.S. 3, 812, 119 (Cl. 260-297), CO. 7d, 21 May 1974, Appl., 878, 914, 21 Nov. (1969), pp. 30.
5. W. Aschwanden, Q. Branacd, E. Kyburz, R. Pfister and (Hoffmann-La Roche, F; und CO A.G.), Eur. Pat. Appl. Ed 56, 617 (Cl. CO 7D 295/02, 28 July 1982, CH Appl., 811273, 16 Jan. 1981, pp. 30.
6. P. Dostert, E. Kyburz (Hoffmann-La Roche, F., und. CO, A.G), Ger. offen. 2, 150, 079 (Cl. CO 7 Cd, A 61 K), B Apr. 1972, Swiss Appl., 14, 972/70, 09 Oct. 1970, pp. 55.
7. P.S. Hofmann, A.B. H. Funke, Eur. J. Med. Chem. Ther., **13**, 251 (1976).
8. W. Treibs and H.J. Klin Khammer, Chem. Ber., 84, 671 (1951).
9. K. Teravishi, K. Hamada and H. Watanabe, Mutation Res., **31**, 97 (1975).
10. I.H. Purchase, E. Longstaff, J. Ashby, J.A. Styles, D. Anderson, P.A. Lefeure and F.R. Westwood, Nature, **264**, 624 (1976).
11. B.N. Ames, W.E. Durson, E. Yamousaki and F.D. Lee, Proc. Nat. Sci., USA, **70**, 2281(1973).
12. D.Clive, D. Scott, B.A. Bridges and F.H. Solbels, Elswier, North-Holloud Biomedical Press, pp. 241 (1977).
13. J. McCann and B.N. Ames, The Salmonella Micosome Mutagenecity Test: Predictive Value for Animal Carcinogenicity (Cited after H.H. Hiatt, J.D. Watson and Winston), J.A. Eds, **4**, 1431 (1977).
14. D. Clive, K.O. Tohason, J.F.S. Spector, A.G. Batson and M.M.M. Brown, Mutation Res., **59**, 61 (1979).
15. D. Brusick, *Genetic Risk Estimation in Principles of*

Genetic Toxicology (Plenum Press, New York and London, 1980), Chapter 5, pp. 109 .

16. A. Lwoff, Lysogeny, Bacterial Rev. , **17**,269 (Cited after: Hollaender, A), pp. 258 (1971).
17. H.W. Smith, J. Gen.Microbiol., **8**, 116 (Cited after Holaender, A.; pp. 258 (1971).
18. J. McCann and B.N. Ames; Proc. Nat. Acad. Sci., USA, **73**, 954 (1976).
19. T. Sugimura, S. Sato, M. Nago, T. Yahaqi, T. Matsushina, Y. Seino, M. Takenchi and T. Kawachi, eds. (Univ. of Tokyo Press, Tokyo Univ. Park Press, Baltimore, MD, 1976), pp. 191.
20. P.N. Magee, The Relationship Between Mutagenesis, Carcinogenesis and Teratogenesis (Cited after: D. Scott, B.A. Bridges and F.H. Sobels), (1977), pp. 15
21. A.M.M. Ali, K.A. Ahmed, M.H. Hamoda and A.V. Sharaf, Bull. Fac. of Agric., Cairo Univ., **34**, 693 (1983).
22. A.M.M. Ali, A.K.A. Selim and M.A.A. Abdel-Aal, Egypt J. Microbiol. , **21**, 201 (1986).