## Minimal Statistical Data for Structure–Function Correlations

PAUL N. CRAIG,

*Smith, Kline, and French Laboratories, Philadelphia, Pennsylvania 19101*

CORWIN H. HANSCH,

*Pomona College, Claremont, California 91711*

JAMES W. MCFARLAND,

*Pfizer Medical Research Laboratories, Groton, Connecticut 06340*

YVONNE C. MARTIN,

*Abbott Laboratories, Chemical Pharmacology Department, North Chicago, Illinois 60064*

WILLIAM P. PURCELL,

*University of Tennessee Medical Units, Memphis, Tennessee 38103*

AND RUDOLPH ZAHRADNÍK

*Czechoslovak Academy of Sciences, Prague, Czechoslovakia*

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In recent years, the number of publications which include results of regression analysis has mushroomed. Since there are many formats for presenting such results, and since the statistical parameters employed will vary with the type of analysis, it is sometimes difficult for the reader to assess the significance of the work from the data presented.

Accordingly, we urge that the following data be presented in each paper submitted to this journal which reports Free–Wilson type analyses or Hansch multiple parameter analyses.

**A. Free–Wilson Analyses.**—It is imperative that the complete structure matrix used in the analysis be presented. In addition, the correlation coefficient $r$, or $r^2$, the $F_{k,n}$, and the group constants obtained, are sufficient to enable the reader to assess and to duplicate the work presented. For predictions, m$\mu$, the overall average, should be given.

**B. Hansch Multiple Parameter Analyses.**—A complete table of the compounds studied, the log $1/c$ values (or other biological data) and all parameter values used for every molecule should be listed. It is especially important to explain estimated or calculated values used. The results of each regression analysis should be accompanied by either 90 or 95% confidence intervals for each term, or a $t$ test value for each term should be given. Whenever applicable, the maximum or minimum $\pi$ value (or any other optimal value obtainable by differentiation of a quadratic equation) should be given, together with confidence intervals. [See discussion by Hansch, *et al.*[1]]

$n$ (the number of compounds included in a particular regression), $s$ (the standard deviation), and $r$ or $r^2$ (the correlation coefficient) should also be given for each equation.

In comparing two equations which differ only by one term, the $F$ test should be applied to test the significance of adding the new term to the simpler equation. If compounds are dropped from consideration (for whatever reason), they should be identified.

(1) C. Hansch, A. R. Steward, S. M. Anderson, and R. Bentley, *J. Med. Chem.*, **11**, 1 (1968).

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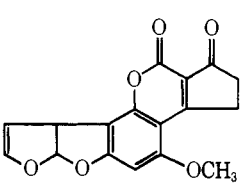
## Synthesis and Toxicology of 5,7-Dibenzyloxycyclopentenone[2,3-*c*]coumarin.[1] A Model Compound of Aflatoxin $B_1$

CHING YUNG WANG AND PAUL F. PARKS*

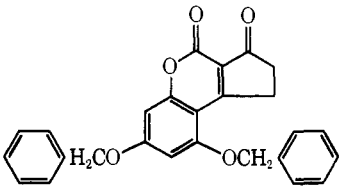
*Department of Animal Science, Agricultural Experiment Station, Auburn University, Auburn, Alabama 36830*

*Received August 31, 1970*

Aflatoxins (aflatoxin $B_1$, I), metabolites of *Aspergillus flavus*, are extremely toxic and carcinogenic to ducklings[2–6] and rats.[7–11] Acute intoxication of aflatoxins produces liver necrosis, degeneration, and bile duct proliferation in ducklings and rats. One model compound of aflatoxin $B_1$, 5,7-dimethoxycyclopentenone[2,3-*c*]coumarin, has been synthesized.[12] We have synthesized 5,7-dibenzyloxycyclopentenone[2,3-*c*]coumarin (II) and determined its toxicity in ducklings and rats.



I



II

The cyclopentenone coumarin moiety of II constitutes a major part of the aflatoxin structure. Compound II, synthesized in an attempt to place a benzene ring in the vicinity of the dihydrofurofuran rings of aflatoxin, was used to study the biological specificity of the dihydrofurofuran ring of aflatoxin $B_1$. The synthesis of II was accomplished in 3 steps. Condensation of phloroglucinol and ethyl methyl $\beta$-ketoadipate yielded 5,7-dihydroxy-4-(2-methoxycarbonylethyl)coumarin, which was cyclized to 5,7-dihydroxycyclopentenone[2,3-*c*]-coumarin. Benzylation of the product gave II.

The acute toxicity of II was determined in 1-day-old Pekin ducklings, and the chronic toxicity and carcinogenicity were studied in weanling rats. Forty-nine

(1) The authors acknowledge the guidance and advice of Dr. C. D. Blanton, Jr., in the synthesis of this compound.

(2) R. Allcroft, in "Mycotoxins in Foodstuffs," G. N. Wogan, Ed., MIT Press, Cambridge, Mass., 1965, p 153.
(3) F. D. Asplin and R. B. A. Carnaghan, *Vet. Rec.*, **73**, 1215 (1961).
(4) W. H. Butler, *Pathol. J. Bacteriol.*, **88**, 189 (1964).
(5) R. B. A. Carnaghan, *Nature (London)*, **208**, 308 (1965).
(6) P. M. Newberne, W. W. Carlton, and G. N. Wogan, *Pathol. Vet.*, **1**, 105 (1964).
(7) J. M. Barnes and W. H. Butler, *Nature (London)*, **202**, 1016 (1964).
(8) W. H. Butler, *Brit. J. Cancer*, **18**, 756 (1964).
(9) S. M. Epstein, B. Bartus, and E. Farber, *Cancer Res.*, **29**, 1045 (1969).
(10) M. C. Lancaster, F. P. Jenkins, and J. McL. Philp, *Nature (London)*, **192**, 1059 (1961).
(11) W. D. Salmon and P. M. Newberne, *Cancer Res.*, **23**, 571 (1963).
(12) T. Asao, G. Buchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, and G. N. Wogan, *J. Amer. Chem. Soc.*, **87**, 882 (1965).

ducklings were treated orally with 50–500 μg of II per duckling biweekly for 2 and 4 weeks. None of the ducklings died within the experimental period. In the other experiment, 5 rats were injected ip with 500 μg of II weekly for 6 consecutive weeks. Again, none of the rats died within the period. Histological examination showed no alteration in the liver, kidney, heart, and brain of the ducklings and rats.

### Experimental Section

**5,7-Dihydroxy-4-(2-methoxycarbonylethyl)coumarin.**—A modification of the procedure of Asao, *et al.*,[12] was used. Phloroglucinol·$2H_2O$ (1.6 g) and 2 g of ethyl methyl β-ketoadipate[13] were mixed with 25 ml of AcOH. Dry HCl was bubbled through the mixt until all of the phloroglucinol was dissolved. The soln stood at room temp overnight. The yellowish needles which formed were collected by filtration. The filtrate, after standing several days, yielded additional needles. The crystals were combined, washed with a small amount of $H_2O$, and dried. Recrystn from MeOH–$H_2O$ gave 2.2 g (84%) of crystals, mp 254–256°. The crude material was used in the next step without further purification.[14]

**5,7-Dihydroxycyclopentenone[2,3-*c*]coumarin.**—The cyclization was carried out by 2 methods: (a) crude 5,7-dihydroxy-4-(2-methoxycarbonylethyl)coumarin was hydrolyzed with aq NaOH, and the product was pptd by the addition of excess HCl soln; heating of this product in Dowtherm A yielded 5,7-dihydroxycyclopentenone[2,3-*c*]coumarin; (b) crude ester (2 g) was suspended in 50 ml of Dowtherm A. The mixt was heated at 253–255° for 1 hr, and after cooling, a greyish ppt formed. The solid was filtered, washed with cyclohexane, and dried. Recrystn from DMF–$H_2O$ yielded 1.2 g of white plates, mp 318–320° dec. *Anal.* ($C_{12}H_8O_5$) C, H, O.[14] The yield from the cyclization reaction was 68%.

**5,7-Dibenzyloxycyclopentenone[2,3-*c*]coumarin (II).**—The ketone (2.2 g) was suspended in 22 ml of THF (distd from Na); NaH (1.5 g) was added, and the mixt was stirred at room temp for 24 hr. THF was removed under reduced pressure, and 22 ml of DMF was added to the solid residue. To the soln was added with stirring 2.3 g of $PhCH_2Cl$, and the mixt was stirred at room temp for 36 hr. The resulting soln was mixed with 25 ml of cold $H_2O$ and extd with $Et_2O$ (4 × 5 ml). Evapn of the combined $Et_2O$ soln produced a greenish yellow solid. This was washed with a small amount of MeOH–$Et_2O$ and dried. The residue was chromatographed on alumina eluting with $CHCl_3$. The first 20 ml of eluate was collected and evapd. The residue was recrystd from $CHCl_3$–MeOH and $CHCl_3$–$Et_2O$ until colorless, silky needles were obtained. The yield was 580 mg (15%), mp 111.5–112°. *Anal.* ($C_{26}H_{20}O_5$) C, H.[14]

(13) E. C. Taylor and A. McKillop, *Tetrahedron*, **23**, 897 (1967).

(14) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Spectral data (ir and uv) were consistent with the proposed structures.

## Synthetic Trypanocides. 2. Substituted 5,6-Dihydro[*c*]benzocarbazoles[1]

JORGE GALLO PECCA AND SEM M. ALBONICO*

*Department of Organic Chemistry, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina*

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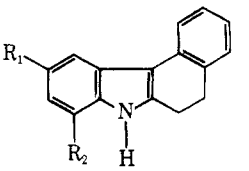
We have previously reported[2] the trypanocidal activity of several substituted 1,2,3,4-tetrahydrocarbazoles. Although the level of *in vitro* activity obtained with some of these compounds was superior to the activity of the dyes[3] clinically used to prevent the infection of the patient by the blood of donors with Chagas-Mazza disease, the search for more active compounds showed that **14** was also more effective than the dyes.

Therefore, a series of substituted 5,6-dihydro[*c*]-benzocarbazoles (DHBC) was prepared (Tables I and II). The DHBC's have been little studied, and none of the substituted compounds in which we are interested have been previously prepared. They fulfill the stability requirement already described,[2] and the acute toxicity is low. No deaths resulted when doses of 1000 mg/kg of the more active compds **29** and **32** were given to white mice either orally or ip. This dose is $10^4$-fold more than is usually required in an average blood transfusion.[4] Furthermore, it was established that in compds containing an N-substituted indole nucleus, Cl and MeO substituents in the benzenoid portion of the aromatic ring provide higher activities against *Trypanosoma cruzi*.

**Chemistry.**—The DHBC's were prepared by the Fisher indole synthesis and by a modified procedure described in the Experimental Section. The unsubstituted DHBC has been previously prepared by Ghigi[5] in low yield and purity; however, under our conditions the yield was 80%. A series of 10-substituted DHBC's were prepared in good yields from 4-substituted phenylhydrazines; under the same conditions, the 2-substituted phenylhydrazines gave a poor yield or none of the desired DHBC. Such a difference in reactivity in the Fisher reaction between 4- and 2-substituted phenylhydrazines has been previously reported by

TABLE I

SUBSTITUTED 5,6-DIHYDRO[*c*]BENZOCARBAZOLES[a]




| No. | $R_1$ | $R_2$ | % yield | Mp, °C | Formula |
|---|---|---|---|---|---|
| 1[b] | H | H | 80 | 102–103 | $C_{16}H_{13}N$ |
| 2 | F | H | 50 | 148–150 | $C_{16}H_{12}FN$ |
| 3 | Cl | H | 85 | 135–136 | $C_{16}H_{12}ClN$ |
| 4 | Br | H | 60 | 158–160 | $C_{16}H_{12}BrN$ |
| 5 | I | H | 60 | 142–144 | $C_{16}H_{12}IN$ |
| 6 | $CH_3$ | H | 60 | 143–145 | $C_{17}H_{15}N$ |
| 7 | $OCH_3$ | H | 96 | 154–155 | $C_{17}H_{15}NO$ |
| 8[c] | H | F | 45 | 143–145 | $C_{16}H_{12}FN \cdot C_6H_3N_3O_7$ |
| 9 | H | Cl | 50 | 105–106 | $C_{16}H_{12}ClN$ |
| 10[d] | H | Br | 40 | 142–144 | $C_{16}H_{12}BrN$ |
| 11 | H | I | 50 | 161–163 | $C_{16}H_{12}IN$ |
| 12[e] | H | $CH_3$ | 60 | 146–147 | $C_{17}H_{15}N$ |
| 13[f] | H | $OCH_3$ | 50 | 140–142 | $C_{17}H_{16}NO$ |

[a] All compds were analyzed for C, H, N, and the anal. results obtained for these elements were within 0.3% of the theoretical values. The uv, ir, nmr, and mass spectra also were in agreement with the proposed structures. Melting points were taken in capillaries and are uncorrected. All compds except **10, 12,** and **13** were recrystd from EtOH. [b] Lit. 97–98°; see E. Ghigi, *Gazz. Chim. Ital.*, **60**, 194 (1930). [c] This was an oil and was analyzed as the picrate which was recrystd from EtOH. [d] Sublimed (135°, 100 μ). [e] Sublimed (120°, 50 μ). [f] Sublimed (100°, 50 μ).

(1) This investigation was supported by grants from the Instituto Nacional de Farmacologia y Bromatologia and C.N.I.C.T. (Argentina).

(2) J. Gallo Pecca and S. M. Albonico, *J. Med. Chem.*, **13**, 327 (1970).

(3) J. Kloetzel, *Rev. Inst. Med. Trop. Sao Paulo*, **3**, 254 (1961).

(4) G. C. Vilaseca, J. A. Cerisola, J. A. Olarte, and A. Zothner, *Vox Sang.*, **11**, 711 (1966).

(5) E. Ghigi, *Gazz. Chim. Ital.*, **60**, 194 (1930).