Notes

Bun-ichi Tamaoki: Analysis of the Double Interaction in Biological Assay, Using the Factorial Coefficients.

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In the pharmacological evaluation of the antispasmodic preparations[1], using a 6-point parallel line assay design, it happened to be necessary to analyse the double interaction in detail, in order to obtain further informations on subdivided interactions.

The general method for this kind of analysis has been well-introduced by various workers[2,3], and is applied to the biological assay, described in this paper, using the special factorial coefficients in the field of study.

The experimental design for this assay made by Dr. K. Takagi, *et al.*, could be expressed in abstract[4] by the following formula:

$$\underbrace{(A_1+A_2+A_3+P_1+P_2+P_3)}_{\mathbf{P}}\ \underbrace{(D_1+D_2+D_3+D_4+D_5+D_6)}_{\mathbf{D}}\ \underbrace{(B_1+B_2+B_3+B_4)}_{\mathbf{B}}$$

Now, for example, the sum of the values in each **B** (or bath) in respect to D_1A_1 is expressed as x_{11}, D_2A_1 as x_{21} and so on, from which Table I can be obtained.

In a similiar way, so that the main effect **P** (or *Treatment* according to their expression[1]) can be divided into five factors, such as *Preparation, Regression, Parallelism, Curvature,* and *Difference of Curvatures,* the double interaction $\mathbf{D}\times\mathbf{P}$ or between *Day* and *Treatment* (P) seemed possible to be analysed also into the interactions between Day and the above stated five factors, which should give more useful information to further assay.

TABLE I Design of the Experiment

$k=4$		**P** A_1	A_2	A_3	P_1	P_2	P_3
D	D_1	x_{11}	x_{12}	x_{13}	x_{14}	x_{15}	x_{16}
	D_2	x_{21}	x_{22}	x_{23}	x_{24}	x_{25}	x_{26}
	⋮	⋮	⋮	⋮	⋮	⋮	⋮
	D_6	x_{61}	x_{62}	x_{63}	x_{64}	x_{65}	x_{66}
Total		$x_{\cdot1}$	$x_{\cdot2}$	$x_{\cdot3}$	$x_{\cdot4}$	$x_{\cdot5}$	$x_{\cdot6}$

Log Doses of A_1 and P_1 are respectively arithmetic series with the same interval.

TABLE II Factorial Coefficients for a 6-point Assay

	A_1	A_2	A_3	P_1	P_2	P_3
Preparation	1	1	1	−1	−1	−1
Regression	1	0	−1	1	0	−1
Parallelism	1	0	−1	−1	0	1
Curvature	1	−2	1	1	−2	1
Difference of Curvatures	1	−2	1	−1	2	−1

In compliance with this pharmacological interests, Sum of Squares of *Day* × *Preparation, Day* × *Regression, etc.,* can be calculated by the following procedures, using the factorial coefficients, as shown in Table II[5].

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* Hongo, Tokyo (玉置文一).
1) K. Takagi, Y. Kasuya, Y. Ota: J. Pharm. Soc. Japan, **73**, 307 (1953).
2) W. G. Cochran, G. M. Cox: "Experimental Designs", J. Wiley & Sons, Inc., New York, U.S.A. (1950).
3) O. Kempthorne: "The Design and Analysis of Experiments", J. Wiley & Sons, Inc., New York, U.S.A. (1952).
4) cf. Table of Footnote (1).
5) C. I. Bliss: Quart. J. Pharm. & Pharmacol. **12**., 82, 182 (1939); J. H. Burn, D. J. Finney, L. G. Goodwin: "Biological Standardization", 2nd Rev. Ed., Oxford Univ. Press, England (1950); D. J. Finney: "Statistical Method in Biological Assay", C. Griffin & Co., England (1952).

1) Interaction between *Day* and *the Difference of the two Preparations, i. e., Day × Prep.*

As shown in Table III, Pr_1 means the difference of the response between the standard and test preparations on D_1 (Day) and therefore, the variation caused by the difference among $Pr_1 \cdots\cdots Pr_6$ is namely one of the interaction in question. Divisor is calculaled as $4\{1^2+1^2+1^2+(-1)^2+(-1)^2+(-1)^2\}=24$, and then the Sum of Squares of 〔*Day × Prep.*〕 can be calculated as follows:

$$S_{Day \times Prep.} = (Pr_1^2+Pr_2^2+\cdots\cdots+Pr_6^2)/24 - Pr\cdot^2/24\times 6 \qquad (f=5)$$

cf.

$$S_{Prep.} = Pr\cdot^2/24\times 6 \qquad (f=1)$$

TABLE III Table for the Calculation of the Interaction, *Day × Prep.*

	Sum of Products
D_1	$(x_{11}+x_{12}+x_{13})-(x_{14}+x_{15}+x_{16})=Pr_1$
D_2	$(x_{21}+x_{22}+x_{23})-(x_{24}+x_{25}+x_{26})=Pr_2$
$\vdots$	$\vdots$
D_6	$(x_{61}+x_{62}+x_{63})-(x_{64}+x_{65}+x_{66})=Pr_6$
Total	$Pr\cdot=Pr_1+Pr_2+\cdots\cdots+Pr_6$ $=(x_{\cdot1}+x_{\cdot2}+x_{\cdot3})-(x_{\cdot4}+x_{\cdot5}+x_{\cdot6})$

TABLE IV Table for the Calculation of the Interaction, *Day × Reg.*

	Sum of Products
D_1	$(x_{11}+x_{14})-(x_{13}+x_{16})=r_1$
D_2	$(x_{21}+x_{24})-(x_{23}+x_{26})=r_2$
$\vdots$	$\vdots$
D_6	$(x_{61}+x_{63})-(x_{63}+x_{66})=r_6$
Total	$r\cdot=r_1+r_2+\cdots\cdots+r_6$ $=(x_{\cdot1}+x_{\cdot4})-(x_{\cdot3}+x_{\cdot6})$

2) Interaction between *Day* and *Regression, i. e., Day × Reg.*

In this case, "Divisor" is $4\{1^2+1^2+(-1)^2+(-1)^2\}=16$, and then,

$$S_{Day \times Reg.} = (r_1^2+r_2^2+\cdots\cdots+r_6^2)/16 - r\cdot^2/16\times 6 \qquad (f=5)$$

cf.

$$S_{Reg.} = r\cdot^2/16\times 6$$

3) Interaction between *Day* and *Parallelism* of the two regression lines, *i. e., Day × Parallelism.*

Similiar to 2), "Divisor" is also 16, then,

$$S_{Day \times Parallelism} = (Pa_1^2+Pa_2^2+\cdots\cdots+Pa_6^2)/16 - Pa\cdot^2/16\times 6 \qquad (f=5)$$

cf.

$$S_{Parallelism} = Pa\cdot^2/16\times 6$$

TABLE V Table for the Calculation of the Interaction, *Day × Parallelism*

	Sum of Products
D_1	$(x_{11}+x_{16})-(x_{13}+x_{14})=Pa_1$
D_2	$(x_{21}+x_{26})-(x_{23}+x_{24})=Pa_2$
$\vdots$	$\vdots$
D_6	$(x_{61}+x_{66})-(x_{63}+x_{64})=Pa_6$
Total	$Pa\cdot=Pa_1+Pa_2+\cdots\cdots+Pa_6$ $=(x_{\cdot1}+x_{\cdot6})-(x_{\cdot3}-x_{\cdot4})$

TABLE VI Table for the Calculation of the Interaction, *Day × Curv.*

	Sum of Products
D_1	$(x_{11}+x_{13}+x_{14}+x_{16})-2(x_{12}+x_{15})=C_1$
D_2	$(x_{21}+x_{23}+x_{24}+x_{26})-2(x_{22}+x_{25})=C_2$
$\vdots$	$\vdots$
D_6	$(x_{61}+x_{63}+x_{64}+x_{66})-2(x_{62}+x_{65})=C_6$
Total	$C\cdot=C_1+C_2+\cdots\cdots+C_6$ $=(x_{\cdot1}+x_{\cdot3}+x_{\cdot4}+x_{\cdot6})-2(x_{\cdot2}+x_{\cdot5})$

4) Interaction between *Day* and *Curvature* of the two log dose-response curves, *i. e., Day × Curv.*

In this case, "Divisor" is $4\{1^2+(-2)^2+1^2+1^2+(-2)^2+1^2\}=48$, and therefore,

$$S_{Day \times Curv.} = (C_1^2+C_2^2+\cdots\cdots+C_6^2)/48 - C\cdot^2/48\times 6$$

cf.

$$S_{Curv.} = C\cdot^2/48\times 6$$

5) Interaction between *Day* and the *Difference of the Curvatures* of the two log dose-response curves, *i. e., Day × Diff. Curv.*

Similiar to 4), "Divisor" is 48, and therefore,

$$S_{Day \times Diff. Curv.} = (Q_1^2 + Q_2^2 + \cdots\cdots + Q_6^2)/48 - Q_{\cdot}^2/48 \times 6 \quad (f=5)$$

cf.

$$S_{Diff. Curv.} = Q_{\cdot}^2/48 \times 6$$

As stated above, the interaction between **B** (or *Bath*) and **P**(or Treatment) can also be analyzed into subdivided ones, if necessary.

TABLE VII Table for the Calculation of the Interaction, *Day×Diff. Curv.*

	Sum of Products
D_1	$(x_{11}+2x_{15}+x_{13})-(x_{14}+2x_{12}+x_{16})=Q_1$
D_2	$(x_{21}+2x_{25}+x_{23})-(x_{24}+2x_{22}+x_{26})=Q_2$
⋮	⋮
D_6	$(x_{61}+2x_{65}+x_{63})-(x_{64}+2x_{62}+x_{66})=Q_6$
Total	$Q_{\cdot}=Q_1+Q_2+\cdots\cdots+Q_6$ $=(x_{\cdot 1}+2x_{\cdot 5}+x_{\cdot 3})-(x_{\cdot 4}+2x_{\cdot 2}+x_{\cdot 6})$

Tatsuo Ohta and Toshio Miyazaki: Furoquinolines. II.[1] Catalytic Reduction of Skimmianine (Addendum).

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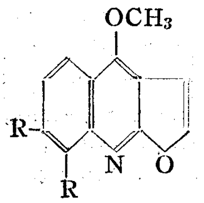
Recently, one of the authors (Ohta)[1] published that 2:3-furo-(2′:3′)-quinoline series of alkaloids, such as dictamnine (I) and skimmianine (II), are cleaved to 2-hydroxy-3-ethylquinoline compounds by catalytic hydrogenation with PtO_2 as a catalyst, and this is a new degradation procedure for the determination of chemical structures employing a small amount of sample.

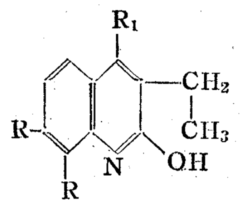
In the present paper, the authors describe the demethylation of the reduction product of skimmianine, namely, 4,7,8-trimethoxy-2-hydroxy-3-ethylquinoline (III). By boiling (III) with conc. hydrochloric acid, hydrolysis of the one methoxyl group in the pyridine nucleus[2] occurred and 7,8-dimethoxy-2,4-dihydroxy-3-ethylquinoline (IV) was formed as crystals of m.p. 202°. The acetylation of (IV) with acetic anhydride containing a few drops of pyridine gave a monoacetate of m.p. 174°. It was presumed that the monoacetate thus obtained is the ester of 4-hydroxyl group, viz. 4-acetoxy-7,8-dimethoxy-2-hydroxy-3-ethylquinoline (V), as in the case of the monoacetate of 2,4-dihydroxyquinoline[3] and 2,4-dihydroxy-3-ethylquinoline[1].

The demethylation of 4,7,8-trimethoxy-2-hydroxy-3-ethylquinoline (III) with HI gave 2,4,7,8-tetrahydroxy-3-ethylquinoline (VI), m.p. 243~244°, which forms a triacetate of m.p. 264° by means of acetic anhydride and pyridine. This acetate was considered as 4,7,8-triacetoxy-2-hydroxy-3-ethylquinoline (VII) from the case of (V).

Analyses were made by Mr. T. Kaneko to whom the authors' thanks are due.



(I) R=H
(II) R=OCH_3



(III) R=OCH_3, R_1≠OCH_3
(IV) R=OCH_3, R_1=OH
(V) R=OCH_3, R_1=$OCOCH_3$
(VI) R=OH, R_1=OH
(VII) R=$OCOCH_3$, R_1=$OCOCH_3$.

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1) Part I: T. Ohta; J. Pharm. Soc. Japan, 73, 63 (1953).
2) Y. Asahina, M. Inubuse: Ber., 63, 2052 (1930).
3) K. Tomita: J. Pharm. Soc. Japan, 72, 1100 (1951); J. N. Ashley, W. H. Perkin, Jr., R. Robinson: J. Chem. Soc., 1930, 388.