The authors express their deep gratitudes to Dr. K. Takeda, Director of Shionogi Research Laboratory, Osaka for ORD measurements. The authors are also indebted to Mr. S. Shimokawa for NMR measurement and to Mrs. T. Toma, Miss A. Maeda for the elemental analysis.

Summary

The roots of *Metaplexis japonica* MAKINO has proved to contain a glycoside mixture. The acidic hydrolysates of the crude glycoside showed the presence of metaplexigenin and two new aglycones. The structure of metaplexigenin was determined.

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34. Satoshi Toki, Reiko Yamasaki, and Teruko Wakiya : Effect of Phenobarbital Derivatives on the Duration of Hexobarbital Hypnosis.

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Recently it was found[1~4] that the activity of drug-metabolizing enzymes in liver microsomes is increased markedly by the administrations of many drugs and carcinogenic polycyclic hydrocarbons, and this phenomenon has attracted the attention of numerous investigators. Some differences were observed[3] between these two kinds of compounds concerning the ability of stimulation on drug metabolism : for example, drugs such as phenobarbital, barbital, orphenadrine, phenylbutazone and aminopyrine activate the metabolism of hexobarbital, aminopyrine, phenylbutazone, zoxazolamine, 3-methyl-4-methylaminoazobenzene and benzo[*a*]pyrene, but polycyclic hydrocarbons such as benzo[*a*]pyrene and 3-methylcholanthrene stimulate the activity of enzymes which metabolize zoxazolamine, 3-methyl-4-methylaminoazobenzene and benzo[*a*]pyrene. Among the above stimulators, phenobarbital exhibited the most potent activity to the metabolism of most of drugs.

It was also observed[3] that increases in enzyme activity are paralleled by an accelerated drug metabolism in the intact animal and by a shortened duration of drug action.

In the present investigation, phenobarbital derivatives in which either *m*- or *p*-position of the benzene ring was substituted by nitro, amino, dimethylamino, diethylamino, hydroxy, methoxy, chloro, or fluoro group, were synthesized and used together with phenobarbital as a stimulating agent. In order to study a relationship between the structure of phenobarbital derivatives and the activating effect of these compounds on the drug metabolism, the potentiating activities were compared measuring the hexobarbital sleeping time in rats and the hexobarbital metabolism in rat liver.

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1) A. H. Conney, J. J. Burns : Nature, **184**, 363 (1959).
2) A. H. Conney, J. R. Gillette, J. K. Inscoe, E. R. Trams, H. Posner : Science, **130**, 1478 (1959).
3) A. H. Conney, C. Davison, R. Gastel, J. J. Burns : J. Pharmacol. Exptl. Therap., **130**, 1 (1960).
4) H. Remmer : Arch. Exptl. Pathol. Pharmakol., **235**, 279 (1959).

Materials and Experimentals*2

Materials——Hexobarbital (5-cyclohexenyl-3, 5-dimethylbarbituric acid) was supplied by Dainippon Pharmaceutical Co. Ltd., and α-3-hydroxyhexobarbital (α-5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid) was prepared by the method of Yoshimura.[5] Phenobarbital (5-ethyl-5-phenylbarbituric acid) and prominal (1-methyl-5-ethyl-5-phenylbarbituric acid) were obtained commercially. Metahydroxy phenobarbital (5-ethyl-5-(*m*-hydroxyphenyl)barbituric acid) was prepared according to the procedure described before.[6]

Preparation of Substituted Phenobarbitals——Following compounds were synthesized after the methods given in the literature : *m*-nitrophenobarbital (5-ethyl-5-(*m*-nitrophenyl)barbituric acid),[7~10] *p*-nitroxyphenobarbital (5-ethyl-5-(*p*-nitrophenyl)barbituric acid),[7,9,10] *m*-aminophenobarbital (5-(*m*-aminophenyl)-5-ethylbarbituric acid),[7~10] *p*-aminophenobarbital (5-(*p*-aminophenyl)-5-ethylbarbituric acid),[7~10] *p*-hydroxyphenobarbital (5-ethyl-5-(*p*-hydroxyphenyl)barbituric acid),[10] *m*-chlorophenobarbital (5-(*m*-chlorophenyl)-5-ethylbarbituric acid).[7]

i) *m*-Dimethylaminophenobarbital (5-(*m*-dimethylaminophenyl)-5-ethylbarbituric acid) : One gram of *m*-nitrophenobarbital (m.p. 274~277.5°) was suspended in 15 ml. of EtOH and 1 ml. of 37% HCHO with 0.22 g. of 10.7% Pd-C catalyst. The reduction was carried out at 15°, and the theoretical amount of hydrogen was absorbed in 2.5 hr. After filtration, the filtrate was evaporated to dryness and the residue was recrystallized repeatedly from EtOH. White crystalline material was obtained. Yield 0.5 g. (49.6%), m.p. 172~173°. *Anal.* Calcd. for $C_{14}H_{17}O_3N_3$: C, 60.95; H, 6.21; N, 15.24. Found : C, 61.06; H, 6.35; N, 14.98.

ii) *p*-Dimethylaminophenobarbital (5-(*p*-dimethylaminophenyl)-5-ethylbarbituric acid) : This compound was synthesized in essentially the same manner as *m*-derivative. To a solution of 5 g. of *p*-nitrophenobarbital (m.p. 191~207°) in 75 ml. of EtOH and 4.5 ml. of 37% HCHO, 1.02 g. of 11.8% Pd-C was added and the mixture was shaken for 5.5 hr. at 15°.

The yellow colored product was dissolved in acetone, and purified by alumina chromatography. The solvent was removed and the residue was recrystallized from EtOH. Yield 0.9 g. (18%), m.p. 243.5~244.5°. *Anal.* Calcd. for $C_{14}H_{17}O_3N_3$: C, 60.95; H, 6.21; N, 15.24. Found : C, 60.92; H, 6.33; N, 14.83.

iii) *m*-Diethylaminophenobarbital (5-(*m*-diethylaminophenyl)-5-ethylbarbituric acid) : One gram of *m*-nitrophenobarbital was suspended in 15 ml. of EtOH and 1 ml. of 80% CH_3CHO, and reduced with hydrogen in the presence of 0.28 g. of 10.7% Pd-C at 20°. The reduction was completed in 5 hr. After filtration, the filtrate was evaporated to dryness and the residue was dissolved in AcOEt. The solution was washed with water, the solvent removed and the residue was recrystallized from EtOH. Yield 0.2 g. (18%) m.p. 137~138°. *Anal.* Calcd. for $C_{16}H_{21}O_3N_3$: C, 63.34; H, 6.97; N, 13.85. Found : C, 63.42; H, 6.93; N, 13.66.

iv) *p*-Diethylaminophenobarbital (5-(*p*-diethylaminophenyl)-5-ethylbarbituric acid) : The preparation of this compound was virtually identical with that described for *m*-analogue. To a solution of 1 g. of *p*-nitrophenobarbital in 15 ml. of EtOH and 1 ml. of 80% CH_3CHO, 0.28 g. of 10.7% Pd-C was added and the mixture was shaken for 7 hr. at 14°. The product was purified by alumina chromatography and recrystallized from acetone. Yield 0.32 g. (29%). m.p. 235~236°. *Anal.* Calcd. for $C_{16}H_{21}O_3N_3$: C, 63.34; H, 6.97; N, 13.85. Found : C, 63.22; H, 6.79; N, 13.75.

v) *p*-Chlorophenobarbital (5-(*p*-chlorophenyl)-5-ethylbarbituric acid) : A solution of cuprous chloride (CuCl) was prepared by adding a mixture of 0.6 g. of $NaHSO_3$, 0.36 g. of NaOH in 4 ml. of water to a solution of 1.6 g. of $CuSO_4$ and 0.8 g. of NaCl in 6 ml. of hot water. The white precipitate of cuprous chloride was separated by decantation, washed once with water and dissolved in 3.7 ml. of conc. HCl.

A diazonium salt solution was prepared by dissolving 2 g. of *p*-aminophenobarbital (m.p. 227~229°) in 2.5 ml. of conc. HCl and 1.2 ml. of water, cooling to 0°, and adding a solution of 0.6 g. of $NaNO_2$ in 2 ml. of water to the amine hydrochloride. The cuprous chloride solution was added to the above diazonium salt solution keeping the temperature between 0° and 5°, and the reaction mixture was stirred 3 hr. at room temperature. After filtration of the solution, orange colored crystal was obtained. The crystal was dissolved in AcOEt, washed with water and the solvent removed. The residue was dissolved again in AcOEt, purified by alumina chromatogaphy and charcoal, and the solvent removed. The colorless residue

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*2 All melting points are uncorrected.

5) H. Yoshimura : This Bulletin, **6**, 13 (1958).
6) H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **3**, 239 (1955).
7) E. W. Bousquet, R. Adams : J. Am. Chem. Soc., **52**, 224 (1930); These workers offer no direct proof of the position of the substituents on the benzene ring.
8) M. M. Rising, J. H. Shroyer, J. Stieglitz : *Ibid.*, **55**, 2817 (1933).
9) A. E. Pierce, M. M. Rising : *Ibid.*, **58**, 1361 (1936).
10) T. C. Butler : J. Pharm. Exptl. Therap., **116**, 326 (1956).

was recrystallized repeatedly from benzene. Yield 1.0 g. (46%), m.p. 190~191°. *Anal.* Calcd. for $C_{12}H_{11}O_3N_2Cl$: C, 54.04; H, 4.16; N, 10.51. Found : C, 54.05; H, 4.20; N, 10.60.

vi) *m*-Fluorophenobarbital (5-ethyl-5-(*m*-fluorophenyl)barbituric acid)*3 : A solution of 1.0 g. of *m*-aminophenobarbital (m.p. 205~209°) in 1 ml. of conc. HCl and 1 ml. of water was diazotized at 0° with 0.3 g. of $NaNO_2$ dissolved in 0.6 ml. of water, and 2.0 ml. of 30% $NaBF_4$ aqueous solution was added. The reaction mixture was stirred for 45 min. and the resulting diazonium fluoroborate was filtered, washed once with 2 ml. of cold 5% $NaBF_4$, once with 2 ml. of cold MeOH and four times with 4 ml. of ether. The dry solid was placed in a flask, heated slowly in an oil bath and decomposed at 105°. The product was extracted with ether, the extract was washed with *N* HCl, then with water, and the ether was removed. After the residue was dissolved in acetone, purified by alumina chromatography and the solvent was removed. The residue was recrystallized repeatedly from 60% EtOH. Yield 0.34 g. (34%), m.p. 175.5~177°.

vii) *p*-Fluorophenobarbital (5-ethyl-5-(*p*-fluorophenyl)barbituric acid)*3 : This compound was prepared from 1.0 g. of *p*-aminophenobarbital and purified by following the above directions concerning the preparation of *m*-fluorophenobarbital. The product was recrystallized from benzene containing small amount of EtOH. Yield 0.19 g. (19%), m.p. 173.5~174.5°.

viii) *m*-Methoxyphenobarbital (5-ethyl-5-(*m*-methoxyphenyl)barbituric acid) : To the suspension of 1.15 g. of *m*-aminophenobarbital hydrochloride in 3.5 g. of glac. AcOH, 0.54 g. of amyl nitrite was added at 13~15° and stirred 2 hr. Stirring was stopped when the reaction mixture changed to pale yellow by adding AcONa, 30 ml. of ether was poured into a vessel and filtered. The separated crystal was suspended in 40 ml. of abs. MeOH and heated for 4 hr. at 120° in an oil bath. After cooling, reaction mixture was filtered, MeOH was distilled off and the residue was extracted with ether. The extract was washed with water, dried on anhyd. Na_2SO_4 and the solvent removed. The reddish brown residue was dissolved in AcOEt, decolorized through an alumina column and the solvent was removed. The residue was recrystallized repeatedly from EtOH. Yield 0.1 g. (9.4%). m.p. 170~171°. *Anal.* Calcd. for $C_{13}H_{14}O_4N_2$: C, 59.53; H, 5.38; N, 10.68. Found : C, 59.70; H, 5.30; N, 10.62.

The melting point of this compound was not depressed by admixture with authentic sample prepared by Tsukamoto, *et al.*[6]

ix) *p*-Methoxyphenobarbital (5-ethyl-5-(*p*-methoxyphenyl)barbituric acid) : By treating 1.06 g. of *p*-aminophenobarbital hydrochloride as described above for the *m*-isomer, diazonium salt was obtained. This compound was placed in a sealed tube with 40 ml. of abs. MeOH and heated for 2 hr. at 55~57° in an oil bath. After reaction, MeOH was removed, the residue was extracted with ether and treated as in the foregoing experiment. Yield 0.4 g. (43%), m.p. 209°. *Anal.* Calcd. for $C_{13}H_{14}O_4N_2$: C, 59.53; H, 5.38; N, 10.68. Found : C, 59.60; H, 5.27; N, 10.79.

Animals——Male Wistar rats weighing 35 to 45 g. were used for all experiments. They were fed a synthetic diet (M-F) which were purchased from Oriental Kobo Kogyo Co. Ltd.

Pretreatment of Rats with Drugs——Phenobarbital and other drugs, which were used in the following pretreatment of rats, were dissolved in 1.1 equivalent of *N* NaOH solution, diluted by 0.9% NaCl soln. and injected intraperitoneally once daily for 2 days. The daily dose was 50 mg./kg. Control rats were injected with 0.9% NaCl soln.

Experiments concerning the measurement of the hexobarbital sleeping time and the enzyme assay were proceeded after 24 hr. from the last injection. The duration of hexobarbital hypnosis was measured by determining when the rats regained their righting reflex after the intraperitoneal injection of 125 mg./kg. of hexobarbital.

Enzyme Assay——The rats were decapitated and livers were removed, pooled, and a 33% liver homogenate was prepared in 0.1*M* phosphate buffer (pH 7.4). The total volume was 6.0 ml. and incubated for 1 hr. at 37°.

After incubation, metabolites were extracted by AcOEt and the quantity of unchanged hexobarbital was determined by spectrophotometrically following the method described by Toki, *et al.*[12]

Results and Discussion

Nitration of phenobarbital produced *m*- and *p*-nitro phenobarbital and the following groups were introduced into either *m*- or *p*-position of the benzene ring by the reduction of the nitro group : amino, dimethylamino and diethylamino groups. Hydroxy-,

*3 It was attempted to identify the both fluoro derivatives by elementary analysis but the experiment was unsuccessful. The existence of fluorine ion in *m*- and *p*-fluorophenobarbital was confirmed by the test with zirconium-alizarin solution.[11]

11) F. Feigl : "Spot test in organic analysis," 5th English Ed. p. 84 (1956) Pub. Elsevier.

12) S. Toki, K. Toki, H. Tsukamoto : This Bulletin, **10**, 708 (1962).

methoxy-, chloro- and fluoro-phenobarbital were prepared from corresponding amino phenobarbital.

The shortening action of these compounds on the duration of hexobarbital hypnosis was examined using rats. The result of this study is shown in Fig. 1. In general,

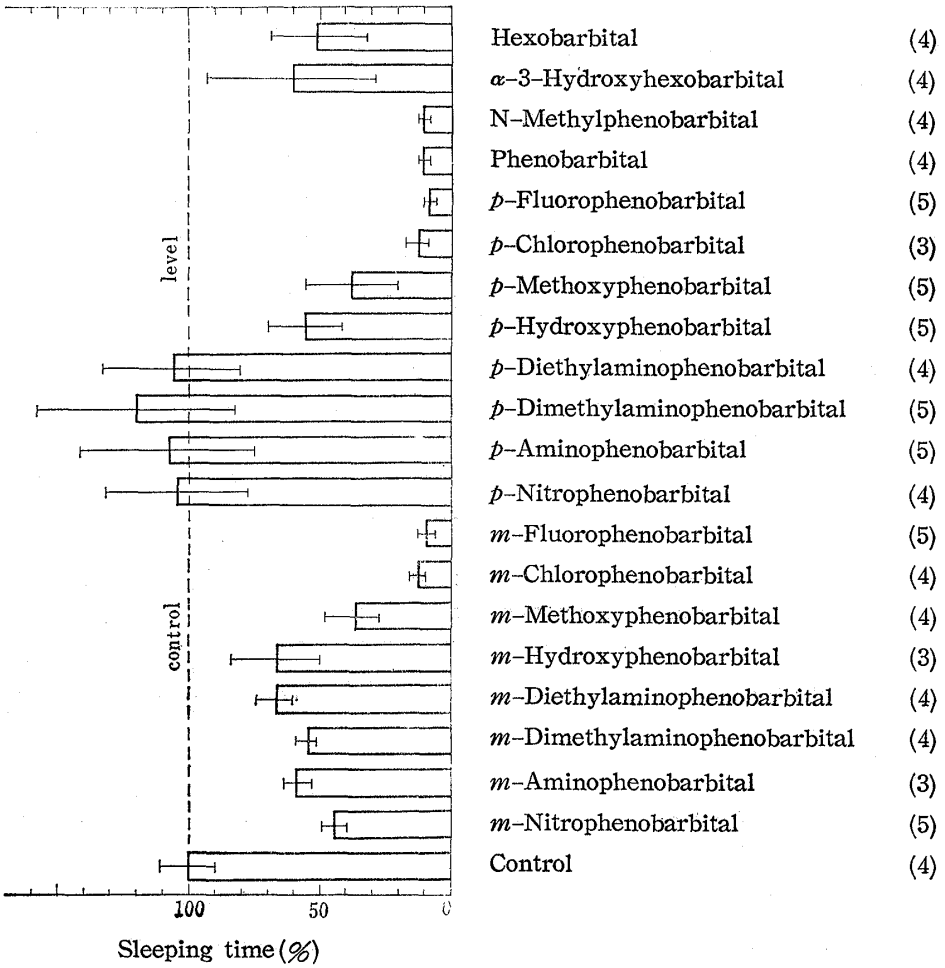

Fig. 1. Effect of Pretreatment of the Phenobarbital Derivatives and Hexobarbitals on the Duration of Hexobarbital Hypnosis

Figures in brackets show the number of animals. The horizontal lines represent the standard deviations. The data are expressed as percentages of sleeping time of control rats which are taken as 100 per cent.

m-derivatives are more potent than *p*-derivatives, of which nitro-, amino-, dimethylamino- and diethylamino-phenobarbital show no shortening effect. Chloro and fluoro derivatives of either substitution exhibited essentially identical and the most potent shortening effect as well as phenobarbital. N-Monomethyl derivative of phenobarbital (prominal) also showed the same shortening effect. Para-hydroxy phenobarbital, the metabolite of phenobarbital,[13] considerably shortened the hexobarbital sleeping time. Its effect, however, was far less than that of phenobarbital. Therefore, it would be clear that shortened duration of hexobarbital hypnosis and induced synthesis of drug-metabolizing enzyme were influenced strongly by phenobarbital pretreatment prior to that it was biotransformed to *p*-hydroxyl derivative. Methylation of *m*- and *p*-hydroxyl derivatives of phenobarbital resulted in the increase in shortening effect on the duration of hexobarbital hypnosis.

13) T. C. Butler : J. Pharmacol. Exptl. Therap., **116**, 326 (1956).

The activating effect of phenobarbital on the liver microsomal metabolism of various drugs, including hexobarbital, has been studied by Remmer,[4] Conney[3] and other workers. It was also demonstrated[3] that pretreatment of animals with phenobarbital markedly shortens the duration of hexobarbital hypnosis. These facts show that the accelerated oxidation of hexobarbital causes a shortened sleeping time.

The metabolic fate of hexobarbital in the rabbit liver has been studied by Toki, *et al.*[12,14,15] and reported that 3-hydroxyhexobarbital was produced by NADPH-dependent microsomal enzyme and this compound was further converted to 3-oxohexobarbital, being catalyzed by NAD- or NADP-dependent enzyme which located in the soluble fraction. In the present experiment concerning the metabolism of hexobarbital by the 9,000×g supernatant fraction of the rat liver, it was observed that the main metabolite was 3-hydroxyhexobarbital, and that 3-oxohexobarbital was very minor metabolite, because rat liver contains only low level of 3-hydroxyhexobarbital dehydrogenase.*4 And it was confirmed that the production of 3-oxohexobarbital was not influenced so much by phenobarbital pretreatment. Therefore, the metabolic rate of hexobarbital *in vitro* was expressed by measuring the amount of unchanged metabolite. The positive relationship between shortened duration of hexobarbital hypnosis and stimulation of its metabolism in the rat liver by phenobarbital derivatives was confirmed by the use of phenobarbital and *m*-chlorophenobarbital as inducer. As shown in Table I, pretreatment of either compound caused about 450 per cent increase in enzyme activity.

Table I. Metabolism of Hexobarbital in Liver of Phenobarbital- and *m*-Chlorophenobarbital-treated Rats

Pretreatment	No. of rats	μMole hexobarbital metabolized/g. liver/hr.	Relative activity
Control	5	0.59	1.0
Phenobarbital	5	2.63	4.46
m-Chlorophenobarbital	5	2.72	4.61

When rats were pretreated with hexobarbital or α-3-hydroxyhexobarbital which is one of the diastereoisomers of 3-hydroxyhexobarbital, the hexobarbital sleeping time was decreased to about a half as that of control (Fig. 1). It is clear that there is no relationship between the hypnotic activity and the shortening activity on the duration of hexobarbital hypnosis, because 3-hydroxyhexobarbital and *p*-hydroxy-, *m*-nitro- and *m*-amino-phenobarbital possessed no hypnotic activity but were able to shorten the duration of hexobarbital hypnosis.

Further studies are now under way to investigate the hypnotic property of synthesized phenobarbital derivatives which are presented here, and to elucidate the induction mechanism of drug-metabolizing enzyme by phenobarbital and other drugs.

Summary

Meta-, and *p*-substituted nitro-, amino-, dimethylamino-, diethylamino-, hydroxy-, methoxy-, chloro- and fluoro-phenobarbital were synthesized. The shortening effect of these compounds, prominal, hexobarbital and α-3-hydroxyhexobarbital on the duration of hexobarbital hypnosis in rats was compared with that of phenobarbital.

*4 Unpublished observation.

14) K. Toki, S. Toki, H. Tsukamoto : J. Biochem. (Tokyo), 53, 43 (1963).
15) K. Toki, H. Tsukamoto : *Ibid.*, 55, 142 (1964).

Meta-, and *p*-chloro- and fluoro-phenobarbital, prominal and phenobarbital showed the most potent shortening effect among above all compounds. *Para*-hydroxyphenobarbital and α-3-hydroxyhexobarbital, the hydroxylated metabolite of phenobarbital and hexobarbital, respectively, were less effective stimulator. *Para*-substituted nitro-, amino-, dimethylamino- and diethylamino-phenobarbital exhibited no stimulation, while corresponding *m*-derivatives and *m*- and *p*-methoxy-phenobarbital showed certain activities.

Pretreatment of rats with *m*-chlorophenobarbital also markedly activated the metabolism of hexobarbital *in vitro*.

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UDC 547.457.03 : 543.422.25

35. Tetsuo Hiraoka, Tadahiro Iwashige, and Issei Iwai : Nuclear Magnetic Resonance Spectral Studies on some Anhydro Furanose Derivatives.

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Recently, many conformational studies on pyranose[1] and furanose[2] derivatives have been made on the basis of proton magnetic resonance spectra. One of the most interesting works in this field has been given by Jardetsky[3] who confirmed that the conformation of D-ribose in purine nucleosides is different from that in pyrimidine nucleosides. These early investigations made use of the Karplus equation[4] or that with modified parameters.[5] However, in the case of the cyclic compounds containing an orbital other

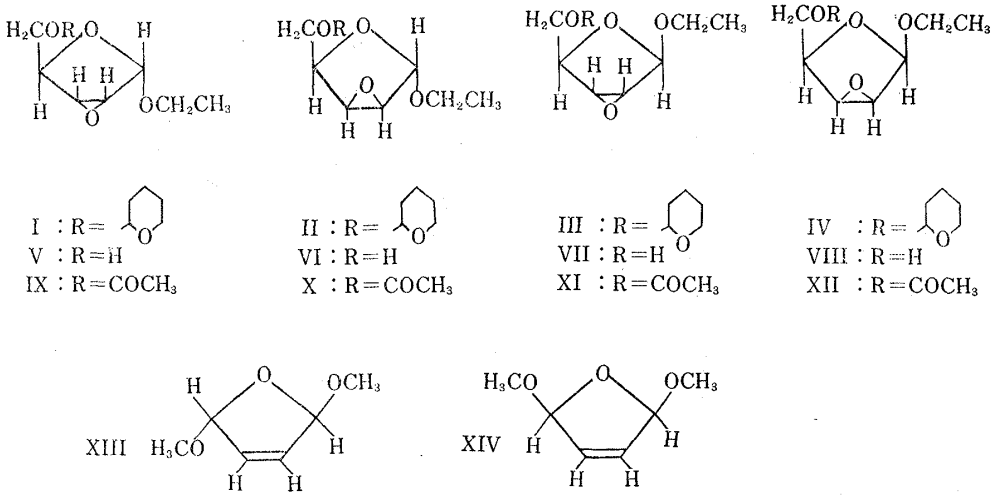

Chart 1.

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1) R. V. Lemieux, *et al.* : J. Am. Chem. Soc., **79**, 1005 (1957); *Idem* : *Ibid.*, **80**, 2237 (1958); *Idem*: *Ibid.*, **80**, 6098 (1958); W. Hofheinz, H. Grisebach : Tetrahedron Letters, **1962**, 377 ; L. D. Hall, L. Hough : Chem. & Ind., **1962**, 1465.
2) R. J. Abraham, *et al* : Chem. & Ind. **1962**, 213.
3) C. D. Jardetzky : J. Am. Chem. Soc., **82**, 229 (1960).
4) M. Karplus : J. Chem. Phys., **30**, 11 (1959).
5) R. J. Abraham, *et al.* : J. Chem. Soc., **1962**, 3699.