**Steroidal 2′-Benzyl-Substituted [16α,17α-*d*]Isoxazolidines 17 and 19.** Compound **10** (0.768 g), *N*-benzylhydroxylamine (0.246 g), and paraformaldehyde (0.04 g) in EtOH (30 mL) was heated under reflux. After 2 days the reaction mixture was poured into $H_2O$, and the precipitate was filtered off, washed with water, and air-dried. This product was chromatographed on silica gel (70 g), eluting with $CHCl_3$/EtOAc (3:1) to give **17** (0.34 g), which was crystallized from $Me_2CO$/hexane to give pure **17** (0.191 g): $^1H$ NMR ($Me_2SO$-$d_6$) δ 0.82 ($C_{10}$ $CH_3$, s), 1.38 ($C_{13}$ $CH_3$, s), 2.06 ($OCOCH_3$, s), 3.37 (3′-H's, m), 3.84 ($CH_2Ph$, d, $J$ = 4 Hz), 4.28 (11-H, m), 4.50 ($C_{21}$ H's, s), 5.91 ($C_4$ H, s), 6.13 ($C_2$ H, dd, $J$ = 10 and 2 Hz), 7.30 ($C_1$ H and phenyl H's, m); $^{13}C$ NMR ($CDCl_3$) δ 17.0 (q, $C_{18}$), 20.5 (q, $C_{19}$), 21.1 (q, $COCH_3$) 30.6 (d, $C_8$) 32.0 ($C_6$, $C_{15}$), 34.2 (t, $C_7$), 40.7 (t, $C_{12}$), 44.2 (s, $C_{10}$) 45.3 (s, $C_{13}$), 47.4 (d, $C_{16}$) 50.8 (d, $C_{14}$), 55.6 (d, $C_9$), 61.9 (t, $NCH_2Ph$), 64.1 (t, $NCH_2$), 67.6 (t, $C_{21}$), 69.8 (d, $C_{11}$), 99.6 (s, $C_{17}$), 122.5 (d, $C_4$), 156.4 (d, $C_1$), 170.0 (s, $COCH_3$), 170.5 (s, $C_5$), 186.5 (s, $C_3$), 205.0 (s, $C_{20}$). Anal. ($C_{31}H_{37}O_6N$) C, H, N.

Similar treatment of **15** (0.804 g) gave **19** (0.26 g), which was crystallized from EtOAc/hexane to give pure **19** (0.197 g): $^1H$ NMR ($Me_2SO$-$d_6$) δ 0.84 ($C_{13}$ $CH_3$, s), 1.50 ($C_{10}$ $CH_3$, s), 2.06 ($OCOCH_3$, s), 3.38 (3′-H's, m), 3.95 ($CH_2Ph$, d, $J$ = 4 Hz), 4.14 (11-H, m), 4.52 ($C_{21}$ H's, s), 6.00 ($C_4$ H, s) 6.10 ($C_2$ H, dd, $J$ = 10 and 2 Hz), 7.28 ($C_1$ H and phenyl H's). Anal. ($C_{31}H_{36}O_6NF$) C, H, N, F.

**Steroidal 2′,3′-Trimethylene-Substituted [16α,17α-*d*]-Isoxazolidine 20.** Pyrroline *N*-oxide (0.204 g) and **10** (0.768 g) in EtOH (30 mL) were heated under reflux for 3 days. A further portion of **6** (0.05 g) was added, and reflux continued for a further 4 h. The reaction mixture was poured into water, and the product was extracted into EtOAc. The organic layer was washed with $H_2O$, dried (anhydrous $Na_2SO_4$), and concentrated under reduced pressure to an oil (0.619 g). Separation of this product by preparative thin-layer chromatography (development solvent $CHCl_3$) gave pure **20** (0.399 g). A portion was crystallized from $CHCl_3$/$Me_2CO$ to give **20**: mp 209–212 °C; $^1H$ NMR ($CDCl_3$) δ 0.94 ($C_{13}$ $CH_3$, s), 1.44 ($C_{10}$ $CH_3$, s), 2.16 ($OCOCH_3$, s), 3.1–3.6 (3′-H's and pyrrolidine H's, m), 4.50 (11-H, m), 4.67 and 4.96 ($C_{21}$ H's, d, $J$ = 18 Hz), 5.99 ($C_4$ H, s), 6.22 ($C_2$ H, dd, $J$ = 10 and 2 Hz), 7.22 ($C_1$ H, d, $J$ = 10 Hz). Anal. ($C_{27}H_{34}O_6N$) C, H, N.

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# A Specific Inhibitor of IgE-Antibody Formation: *n*-Pentyl β-D-Fructopyranoside

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*n*-Pentyl β-D-fructopyranoside significantly suppresses IgE-antibody formation in rats and mice when orally administered, while no formation of hemagglutinin was observed. This is the first compound that is novel in structure and which exhibits a selective inhibition of IgE-antibody formation.

By screening ethanol or water extracts of 20 traditional Chinese crude drugs that had been widely used for diseases caused by allergies to passive cutaneous anaphylaxis (PCA) and passive hemagglutination (PHA), Koda et al. found *Zizyphus fructus* to be one of the most efficient drugs.[1] An earlier experiment indicated that the ethyl α-D-fructofuranoside in the ethanol extract of *Zizyphus fructus* suppressed IgE-antibody formation in rats immunized with the dinitrophenylated *Ascaris* extract (DNP-As).[2] In order to find a potent drug, we undertook the derivatization of alkyl β-D-fructopyranosides as part of the assay of antibody formation. This report will describe how *n*-pentyl β-D-fructopyranoside given either intraperitoneally or orally effectively suppresses IgE-antibody formation in both rats and mice, without any suppression of hemagglutinin formation.

**Synthesis and Characterization of Compounds 1–16.** The compounds in Table I were synthesized by a modification of the method described under Experimental Section. The purity of each alkyl D-fructoside that was separated on chromatography was examined by means of gas chromatographical analysis of the trimethylsilyl derivatives; it was confirmed that each alkyl D-fructoside was 98–99% pure. The ring size and anomeric nature[3] were assigned to the glycosides by means of gas chromatography,[4] mass spectrometry,[5] and $^{13}C$ nuclear magnetic resonance ($^{13}C$ NMR) determination.[6] As a potent tool, $^{13}C$ NMR was effectively applied to the configurational and conformational analyses. The $^{13}C$ NMR spectral examination of C-2 in alkyl D-fructosides clearly shows that C-2 in alkyl D-fructofuranosides resonates at a lower field than that of alkyl D-fructopyranosides. The set of resonances in methyl and ethyl D-fructofuranosides (**5** and **6**) is identified as having the β-D-furanoside form because of the cis interaction of vicinal hydroxy groups at C-2 and C-3, causing an up-field shift of the C-2 resonance relative to the same resonance of alkyl α-D-fructofuranosides (1–4). The set of ring carbons in alkyl β-D-fructopyranosides resonates upfield from the the corresponding set of ring carbons in alkyl D-fructofuranosides, as was demonstrated in D-fructose. As a result of $^{13}C$ NMR spectral studies,

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(1) A. Koda, Y. Yanagihara, H. Nagai, and K. Sakamoto, *Folia Pharmacol. Jpn.*, **69**, 88 (1973).
(2) A. Yagi, A. Koda, N. Inagaki, Y. Haraguchi, K. Noda, N. Okamura, and I. Nishioka, *Yakugaku Zasshi*, **101**, 700 (1981).
(3) S. J. Angyal, *Angew. Chem., Int. Ed. Engl.*, **8**, 157 (1969).
(4) M. Gee and H. G. Walker, Jr., *Anal. Chem.*, **34**, 650 (1962).
(5) (a) K. Heynes and D. Huller, *Tetrahedron*, **21**, 55 (1965); (b) K. Heynes and H. Scharman, *ibid.*, **21**, 509 (1965); (c) H. CH. Curtius, M. Muller, and J. A. Vollmin, *J. Chromatogr.*, **37**, 216 (1968).
(6) (a) D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355 (1970); (b) E. Breitmaier and W. Voelter, "$^{13}C$ NMR Spectroscopy", H. F. Ebel, Ed., Verlag Chemie GmbH, Weinheim/Bergerstr., 1974, p 223; (c) A. S. Perlin, N. Cyr, H. J. Koch, and B. Korsch, *Ann. N.Y. Acad. Sci.*, **222**, 935 (1973); (d) L. Que, Jr., and G. R. Gray, *Biochemistry*, **13**, 146 (1974); (e) W. Funcke and A. Klemer, *Ann. Chem.*, 1232 (1975); (f) W. Funcke and A. Klemer, *Carbohydr. Res.*, **50**, 9 (1976).

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Table I. $^{13}C$ NMR Spectral Data of Alkyl (or Benzyl) D-Fructosides

| no.[a] | | chemical shift from ($Me_4Si$),[b] ppm | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | C-2 | C-3 | C-4 | C-5 | C-1 | C-6 | *O*-alkyl (or benzyl) | | |
| | | | * | | | * | | | | |
| 1 | $CH_3$ | 109.6 | 84.8 | 82.7 | 79.2 | 63.3 | 60.8 | 49.8 | | |
| 2 | $C_2H_5$ | 108.6 | 83.9 | 82.6 | 77.9 | 61.9 | 61.4 | 57.4 | 16.1 | |
| 3 | $n$-$C_3H_7$ | 109.1 | 84.0 | 82.7 | 78.6 | 64.7 | 62.8 | 61.2 | 24.5 | 11.8 |
| 4 | $n$-$C_4H_9$ | 109.3 | 84.1 | 83.0 | 78.7 | 62.9 | 62.9 | 61.6 | 33.6 | 20.8 |
| | | | | | | | | 15.1 | | |
| 5 | $CH_3$ | 104.8 | 82.9 | 78.4 | 76.9 | 64.3 | 60.4 | 49.5 | | |
| 6 | $C_2H_5$ | 104.8 | 82.9 | 78.5 | 77.1 | 64.5 | 61.9 | 57.4 | 16.1 | |
| 7 | $CH_3$ | 102.0 | 72.0 | 71.5 | 71.2 | 65.7 | 63.6 | 49.8 | | |
| 8 | $C_2H_5$ | 101.6 | 71.2 | 70.6 | 69.7 | 64.8 | 62.8 | 57.2 | 15.7 | |
| 9 | $n$-$C_3H_7$ | 102.1 | 71.6 | 71.1 | 70.2 | 65.6 | 64.3 | 63.3 | 24.6 | 11.9 |
| 10 | $i$-$C_3H_7$ | 102.4 | 71.2 | 70.8 | 69.7 | 65.2 | 64.7 | 64.5 | 24.9 | 24.3 |
| 11 | $n$-$C_4H_9$ | 102.3 | 71.8 | 71.3 | 70.3 | 65.6 | 63.4 | 62.4 | 33.6 | 20.9 |
| | | | | | | | | 15.1 | | |
| 12 | $i$-$C_4H_9$ | 101.2 | 71.1 | 70.6 | 69.8 | 68.1 | 64.8 | 62.8 | 29.6 | 19.9 |
| | | | | | | | | 19.6 | | |
| 13 | $n$-$C_5H_{11}$ | 101.4 | 71.4 | 70.8 | 70.4 | 65.0 | 63.3 | 61.7 | 30.7 | 29.5 |
| | | | | | | | | 23.5 | 14.5 | |
| 14 | $i$-$C_5H_{11}$ | 101.4 | 71.3 | 70.8 | 70.4 | 65.0 | 63.3 | 60.1 | 34.0 | 26.1 |
| | | | | | | | | 23.1 | 23.0 | |
| 15 | $n$-$C_6H_{13}$ | 101.4 | 71.4 | 70.8 | 70.4 | 65.0 | 63.3 | 61.7 | 32.8 | 31.0 |
| | | | | | | | | 27.0 | 23.6 | 14.4 |
| 16 | benzyl | 102.0 | 71.1 | 70.5 | 69.8 | 65.1 | 63.7 | 63.0 | 139.3 | 129.0 |
| | | | | | | | | 128.2 | 128.0 | |

[a] 1-4, alkyl α-D-fructofuranosides; 5 and 6, alkyl β-D-fructofuranosides; 7-15, alkyl β-D-fructopyranosides; 16, benzyl β-D-fructopyranoside. An asterisk indicates that these assignments may be reversed. [b] Recorded at 25.5 MHz in $CD_3OD$ with $Me_4Si$ as the internal standard.

Table II. Physical Properties of Alkyl (or Benzyl) D-Fructosides and Their Effects on IgE-Antibody and Hemagglutinin Formations in Rats Immunized with DNP-As and *B. pertussis*[a]

| no. | mp, °C | $[\alpha]_D$, deg | yield, % | PCA ($log_2$)[b] | | PHA ($log_2$)[c] | |
|---|---|---|---|---|---|---|---|
| | | | | titer | control | titer | control |
| 1 | oil | +67.8 | 28.0 | 5.8 ± 0.70 | 7.3 ± 0.40 | | |
| 2 | oil | +62.8 | 9.0 | 6.5 ± 0.60 | 7.3 ± 0.40 | | |
| 3 | oil | +58.5 | 42.0 | 7.3 ± 0.25 | 7.3 ± 0.40 | | |
| 4 | oil | +53.8 | | 6.7 ± 0.50 | 7.3 ± 0.40 | | |
| 5 | oil | −39.6 | 27.2 | | | | |
| 6 | oil | −15.5 | 28.0 | 6.1 ± 0.40 | 7.9 ± 0.47 | | |
| 7 | 115-117 | −142.0 | 42.4 | 7.9 ± 0.40 | 7.9 ± 0.47 | | |
| 8 | 150-151 | −136.0 | 10.0 | 7.1 ± 0.68 | 7.9 ± 0.47 | 7.9 ± 0.37 | 8.2 ± 0.45 |
| 9 | 157-158 | −142.1 | 43.2 | 6.8 ± 0.67 | 7.9 ± 0.47 | 7.9 ± 0.53 | 8.2 ± 0.45 |
| 10 | 111-112 | −134.1 | 10.8 | | | | |
| 11 | 147-149 | −138.1 | 42.4 | 7.2 ± 0.48 | 7.9 ± 0.47 | 8.1 ± 0.58 | 8.2 ± 0.45 |
| 12 | 157-158 | −130.0 | 41.4 | 6.2 ± 0.50* | 7.9 ± 0.47 | 8.0 ± 0.84 | 8.2 ± 0.45 |
| 13 | 129-130 | −123.0 | 35.0 | 5.7 ± 0.50** | 7.9 ± 0.47 | 8.1 ± 0.67 | 8.2 ± 0.45 |
| 14 | 120-122 | −131.0 | 29.1 | | | | |
| 15 | 130-132 | −120.0 | 46.8 | 6.6 ± 0.65 | 7.9 ± 0.47 | 7.3 ± 0.75 | 8.2 ± 0.45 |
| 16 | 160-161 | −124.0 | 15.0 | 7.0 ± 0.47 | 7.9 ± 0.47 | 9.0 ± 0.34 | 8.2 ± 0.45 |

[a] Compounds 1-16 were administered at 100 (mg/kg)/day ip for 5 days from day 0, and the titers of the antibodies were evaluated on day 8. The values are means ± SE obtained from eight rats: * and ** = statistical significance from the control at $p < 0.05$ and $p < 0.01$, respectively. [b] Passive cutaneous anaphylaxis. [c] Passive hemagglutination. Titers are shown in $n$ of the highest dilution ($2^n$).

compounds 7-15 were identified as alkyl D-fructopyranosides with a $^1C_4$ conformation (Table I).

## Biological Results and Discussion

The results obtained from the PCA test showed that *n*-pentyl β-D-fructopyranoside (13) significantly suppressed the formation of the IgE antibody, while no suppression was observed in the formation of the IgM or IgG antibodies, as estimated by means of a PHA test (Table II). However, compounds dissolved in distilled water given po did not suppress the formation of the IgE antibody in rats. This suggests a degradation of alkyl β-D-fructopyranosides by the gastric juice in the stomach. Indeed, alkyl D-fructopyranosides were completely decomposed with artificial gastric juice (pH 1.2) at 37 °C within 48 h. Therefore, alkyl β-D-fructopyranosides were dissolved in a 2% $NaHCO_3$ solution to avoid the degradation when given po. As shown in Tables III and IV, compound 13 again suppressed the formation of the IgE antibody on day 10 after the immunization. Furthermore, this suppression was found to be at its maximum on day 21. On the other hand, alkyl β-D-fructopyranosides exerted no significant suppression of hemagglutinin formation. Compound 13 was not toxic; $LD_{50} > 5$ g/kg in rats.

To examine the dose dependency and the time course of compound 13, we undertook the following experiments in order to investigate the formation of the IgE antibody.

**Study of the Dose Dependency of Compound 13 in Connection with the Formation of the IgE Antibody in Rats.** As shown in Table V, the administration of

Table III. Effect of Alkyl β-D-Fructopyranosides on IgE-Antibody and Hemagglutinin Formations in Female Wistar Rats[a]

| compd | PCA titer ($log_2$)[b] | PHA titer ($log_2$)[c] |
|---|---|---|
| control | 7.4 ± 0.45 | 9.3 ± 0.40 |
| 8 | 6.7 ± 0.44 | 8.5 ± 0.64 |
| 9 | 6.5 ± 0.64 | 8.3 ± 0.43 |
| 11 | 6.4 ± 0.65 | 8.4 ± 0.41 |
| 12 | 6.6 ± 0.47 | 9.1 ± 0.28 |
| 13 | 5.7 ± 0.49* | 9.9 ± 0.33 |
| 14 | 6.5 ± 0.53 | 8.5 ± 0.35 |
| 15 | 6.3 ± 0.60 | 9.1 ± 0.33 |

[a] Rats were immunized with 1 mg of DNP-As and $10^{10}$ *B. pertussis* on day 0 and boosted with 0.5 mg of DNP-As alone on day 5. Serum was collected from each rat on day 8. Compounds **8–15** were dissolved in a 2% $NaHCO_3$ solution and given 100 (mg/kg)/day po for 5 days from day 0. The values are means ± SE from eight rats: * = statistical significance from the control at $p < 0.05$. [b] Passive cutaneous anaphylaxis. [c] Passive hemagglutination. Titers are shown in $n$ of the highest dilution ($2^n$).

**Effect of the Time Course of Compound 13 on the Formation of the IgE Antibody in Mice.** Compound **13** was administered following schedule 1 (Chart I). As shown in Table VI, compound **13**, given in a dose of 100 mg/kg for 5 days before or after the primary immunization, markedly suppressed the IgE antibody formation on day 10, and the administration of the compound after the immunization was more effective than its preadministration. After the secondary immunization, a significant suppression of the IgE-antibody formation on day 45 was found in both pre-non- and pre-preadministration groups. The results of the PHA test are summarized in Table VII. No apparent difference from the control was observed in hemagglutinin formation (Table VII).

Furthermore, the above results were confirmed by another experiment following schedule 2 (Chart II). As shown in Tables VIII and IX, the posttreated group, in which compound **13** was given for 5 days after the first immunization, showed a suppression of IgE-antibody formation on days 20 and 30 in statistically significant amounts (with the control at $p < 0.01$); a statistically significant suppression was also observed on day 43 (with the control at $p < 0.05$). The results of the PHA test are summarized in Table IX. No apparent difference was found in hemagglutinin formation, except for a slight suppression on day 20.

Table IV. Effect of Alkyl β-D-Fructopyranosides on IgE-Antibody and Hemagglutinin Formations in Female BALB/c Mice Immunized with 10 μg of DNP-As and 2 mg of $Al(OH)_3$[a]

| | PCA titer ($log_2$)[b] | | | PHA titer ($log_2$)[c] | | |
|---|---|---|---|---|---|---|
| compd | day 10 | day 21 | day 30 | day 10 | day 21 | day 30 |
| control | 7.7 ± 0.67 | 10.0 ± 0.0 | 8.2 ± 0.17 | 5 | 5 | 6 |
| 8 | 7.9 ± 0.66 | 8.3 ± 0.44** | 7.8 ± 0.25 | 5 | 5 | 6 |
| 9 | 7.7 ± 0.41 | 8.9 ± 0.42* | 8.6 ± 0.16 | 4 | 5 | 6 |
| 11 | 8.0 ± 0.58 | 9.9 | 9.1 | 5 | 5 | 6 |
| 12 | 6.6 ± 0.39 | 9.0 ± 0.32* | 7.4 ± 0.56 | 5 | 5 | 6 |
| 13 | 6.3 ± 0.48 | 8.3 ± 0.60* | 7.9 ± 0.38 | 5 | 5 | 6 |
| 14 | 8.1 ± 0.48 | 8.6 ± 0.56* | 8.8 ± 0.25 | 5 | 5 | 7 |
| 15 | 8.0 | 7.9 | 7.5 | 6 | 5 | 6 |
| 16 | 8.6 ± 0.39 | 9.0 ± 0.32* | 8.4 ± 0.38 | 6 | 5 | 6 |

[a] Mice were immunized with 10 μg of DNP-As and 2 mg of $Al(OH)_3$ on day 0. Compounds **8–16** were dissolved in a 2% $NaHCO_3$ solution and given 100 (mg/kg)/day po for 5 days after the immunization. Serum was collected from each mouse on days 10, 21, and 30 and estimated with the titer of antibodies. The values represent the titer of pooled sera from five mice and are means ± SE: * and ** = statistical significance from the control at $p < 0.05$ and $p < 0.01$. [b] Passive cutaneous anaphylaxis. [c] Passive hemagglutination. Titers are shown in $n$ of the highest dilution ($2^n$).

Table V. Effect of Various Doses of Compound 13 on Antibody Formations in Female Wistar Rats[a]

| compd | dose, mg/kg | PCA titer ($log_2$)[b] | PHA titer ($log_2$)[c] |
|---|---|---|---|
| control | | 6.6 ± 0.15 | 10.5 ± 0.27 |
| 13 | 10 | 6.0 ± 0.34 | 11.3 ± 0.42 |
| | 20 | 6.6 ± 0.32 | 11.0 ± 0.44 |
| | 50 | 6.5 ± 0.22 | 11.2 ± 0.37 |
| | 100 | 5.8 ± 0.26** | 11.1 ± 0.14* |
| | 200 | 5.6 ± 0.24** | 11.3 ± 0.24* |
| CP | 10 | <2 | 1.3 ± 0.42* |

[a] Rats were immunized with 1 mg of DNP-As and $10^{10}$ *B. pertussis* on day 0 and boosted with 0.5 mg of DNP-As alone on day 5. Serum was collected from each rat on day 8. Compound **13** was dissolved in a 2% $NaHCO_3$ solution and given 10 to 200 (mg/kg)/day po for 5 days from day 0. Cyclophosphamide (CP) was dissolved in distilled water and given 10 (mg/kg)/day po for the same period. Each value represents the mean ± SE of from four to eight animals. * and ** = statistical significance from the control at $p < 0.05$ and $p < 0.01$, respectively. [b] Passive cutaneous anaphylaxis. [c] Passive hemagglutination. Titers are shown in $n$ of the highest dilution ($2^n$).

compound **13** in doses of 50–200 mg/kg decreased the PCA titer in a dose-dependent fashion; relatively high doses of 100 and 200 mg/kg also showed significant suppression, while no suppression of the hemagglutinin formation was observed.

Since compound **13** is considered to have no antigenicity, based on its structural nature, the antigen nonspecific inhibitory action on IgE-antibody formation is fully expected. Compound **13** inhibits IgE-antibody formation when administered after the immunization. Therefore, it is certainly possible that compound **13** suppresses the intermittent formation of the IgE antibody.

The findings that *n*-pentyl β-D-fructopyranoside selectively suppressed the formation of the IgE antibody and had a novel structure are of significant interest in an immunopharmacological study. Further studies of alkyl derivatives of aldohexose and aldopentose and of the immunopharmacological mechanism of compound **13** are now in progress.

## Experimental Section

Melting points are uncorrected. Optical rotation was measured in MeOH on a JASCO DIP-SL automatic polarimeter at 22 °C. $^{13}C$ NMR spectra in 0.5 M $CD_3OD$ were recorded on a JEOL FX-100 spectrometer at 22 °C in a 5-mm spinning tube, with $Me_4Si$ as the internal standard (25.5 MHz). FT measurement conditions: spectral width, 47.3 kHz; pulse flipping angle, 45–90°; number of data points, 8192; pulse repetition, 3 s. The mass

Table VI. Effect of Compound 13 on IgE-Antibody Formation at Different Times during the Course of the Primary and/or Secondary Immunization in Female BALB/c Mice[a]

| drug administration | | PCA titer ($log_2$)[b] | | | |
|---|---|---|---|---|---|
| 1st | 2nd | day 10 | day 20 | day 30 | day 45 |
| non | -non | 9.4 ± 0.32 | 8.2 ± 0.21 | 7.6 ± 0.76 | 12.2 ± 0.26 |
| | -pre | | | | 11.2 ± 0.64 |
| | -post | | | | 11.9 ± 0.32 |
| pre | -non | 8.7 ± 0.16* | 8.3 ± 0.19 | 7.9 ± 0.31 | 11.3 ± 0.20* |
| | -pre | | | | 10.6 ± 0.51* |
| | -post | | | | 11.8 ± 0.41 |
| post | -non | 8.2 ± 0.30** | 8.3 ± 0.25 | 7.3 ± 0.48 | 11.4 ± 0.32 |
| | -pre | | | | 11.9 ± 0.51 |
| | -post | | | | 11.7 ± 0.20 |

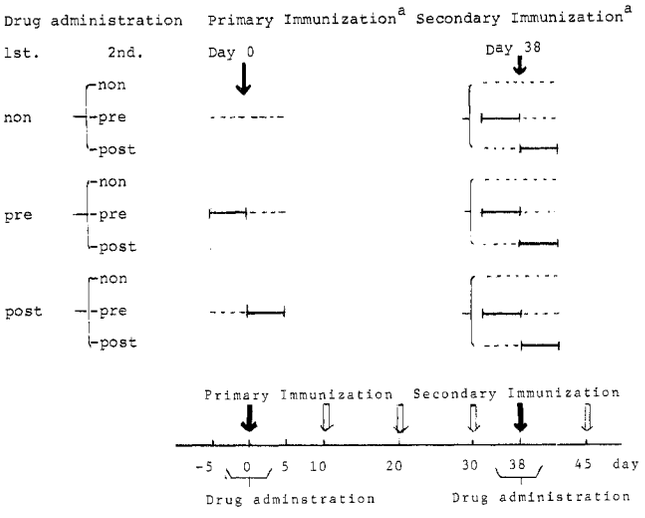
[a] Mice were immunized with 10 μg of DNP-As and 3 mg of $Al(OH)_3$ on days 0 and 38. Serum was collected from each mouse on days 10, 20, 30, and 45. Compound 13 was dissolved in a 2% $NaHCO_3$ solution and given 100 (mg/kg)/day po for 5 days before or after the primary and/or secondary immunization. Each value represents the mean ± SE of from four to eight animals. * and ** = statistical significance from non-non group at $p < 0.05$ and $p < 0.01$, respectively. [b] Passive cutaneous anaphylaxis. Titers are shown in $n$ of the highest dilution ($2^n$).

Table VII. Effect of Compound 13 on Hemagglutinin Formation at Different Times during the Course of the Primary and/or Secondary Immunization in Female BALB/c Mice[a]

| drug administration | | PHA titer ($log_2$)[b] | | | |
|---|---|---|---|---|---|
| 1st | 2nd | day 10 | day 20 | day 30 | day 45 |
| non | -non | 5 | 7 | 5 | 8 |
| | -pre | | | | 7 |
| | -post | | | | 8 |
| pre | -non | 4 | 7 | 5 | 7 |
| | -pre | | | | 7 |
| | -post | | | | 7 |
| post | -non | 5 | 6 | 5 | 7 |
| | -pre | | | | 7 |
| | -post | | | | 7 |

[a] Mice were immunized with 10 μg of DNP-As and 3 mg of $Al(OH)_3$ on days 0 and 38. Serum was collected from each mouse on days 10, 20, 30, and 45. Compound 13 was dissolved in a 2% $NaHCO_3$ solution and given 100 (mg/kg)/day po for 5 days before or after the primary and/or secondary immunization. Each value represents the titer of pooled sera of from four to eight animals. [b] Passive hemagglutination. Titers are shown in $n$ of the highest dilution ($2^n$).

Chart I. Administration Schedule (1) of Compound 13




[a] Mice were immunized with 10 μg of DNP-As and 3 mg of $Al(OH)_3$ on days 0 (⇩) and 38 (⇩). Compound 13 was dissolved in a 2% $NaHCO_3$ solution and given 100 (mg/kg)/day po for 5 days before or after the primary and/or secondary immunization: ⊢⊣ = drug administration for 5 days; non = nontreated, pre = preadministration; post = postadministration. Serum was collected from each mouse on days 10, 20, 30, and 45 (⇩) to estimate antibody titers.

spectral data were recorded on a JEOL-D 100 mass spectrometer with an accelerating potential of 30 eV and a source temperature at 170 °C. GLC analysis employing a Shimazu GC-4BM apparatus was carried out on the permethyl ethers (condition A) and on the $Me_3Si$ ether (condition B) of alkyl D-fructosides. Condition A: column, 5% 1,4-butanediol succinate 3 mm × 2 m, glass; Shimalite W (60–80 mesh); column temperature, 175 °C; detector temperature, 220 °C; carrier gas, $N_2$ at 40 mL/min. Condition B: column, 1.5% SE 30, 3 mm × 2 m, glass; Chromosorb W (AW-DMCS, 60–80 mesh); column temperature, 160 °C; detector temperature, 270 °C; carrier gas, $N_2$ at 40 mL/min; column, 2% OV 17, 3 mm × 2 m glass column; Chromosorb W (AW-DMCS, 60–80 mesh); column temperature, 200 °C; carrier gas, $N_2$ at 40 mL/min.

**Synthesis.** Alkyl D-fructosides were prepared in good yields by a modification of the standard method.[7] In a typical experiment, dried D-fructose (10 g) was suspended in $n$-pentyl alcohol (485 mL) with 0.1% hydrogen chloride, and the mixture was stirred for 20 h at room temperature. The reaction mixture was then neutralized with 25% $NH_4OH$, after the precipitate has been filtrated off, and the filtrate was concentrated to a syrup. The syrup, containing configurational and conformational isomers, was chromatographed over a silica gel column, with EtOAc saturated with $H_2O$ or $CHCl_3$–EtOH–$H_2O$ (7:3:1) as a solvent, to give 3.5 g of $n$-pentyl glycoside (13) in a pure β-D-fructopyranoside form. In the same way, the formation of methyl or ethyl glycosides was undertaken to yield a mixture of configurational and conformational isomers, each of which was purified by means of silica gel chromatography.

**Biological Procedures.** Female Wistar rats weighing 190–210 g and 7–8 week-old female BALB/C mice were used for the formation of antibodies. Throughout the experiments, DNP-As was used as an antigen. The preparation of DNP-As was as follows. An extract from *Ascaris suum*, prepared by the method of Strejan and Campbell,[8] was dinitrophenylated with 2,4-dinitrobenzenesulfonic acid by the method of Eisen et al.[9] The reaction mixture was then dialysated to remove any uncoupled reagent, lyophilized, and stored at −20 °C. The protein content of the antigen, as measured by the microbiuret method,[10] was

(7) C. P. Barry and J. Honeyman, *Adv. Carbohydr. Chem.*, 7, 53 (1952).

(8) G. Strejan and D. H. Campbell, *J. Immunol.*, **101**, 628 (1968).
(9) H. N. Eisen, S. Belman, and M. E. Carsten, *J. Am. Chem. Soc.*, **75**, 4583 (1953).
(10) R. F. Itzhaki and D. M. Gill, *Anal. Biochem.*, **9**, 401 (1964).

Table VIII. Effect of Compound 13 on IgE-Antibody Formation at Different Times during the Course of the Primary and/or Secondary Immunization in Female BALB/c Mice[a]

| drug administration | | PCA titer ($log_2$)[b] | | | |
|---|---|---|---|---|---|
| 1st | 2nd | day 10 | day 20 | day 30 | day 43 |
| non | -non | 5.8 ± 0.36 | 6.6 ± 0.29 | 7.2 ± 0.30 | 10.8 ± 0.29 |
| pre | -pre | 5.7 ± 0.21 | 6.0 ± 0.22 | 6.5 ± 0.32 | 10.2 ± 0.23 |
| | -post | | | | 10.4 ± 0.33 |
| post | -pre | 5.1 ± 0.25 | 5.1 ± 0.26** | 5.5 ± 0.31** | |
| | -post | | | | 9.8 ± 0.24* |

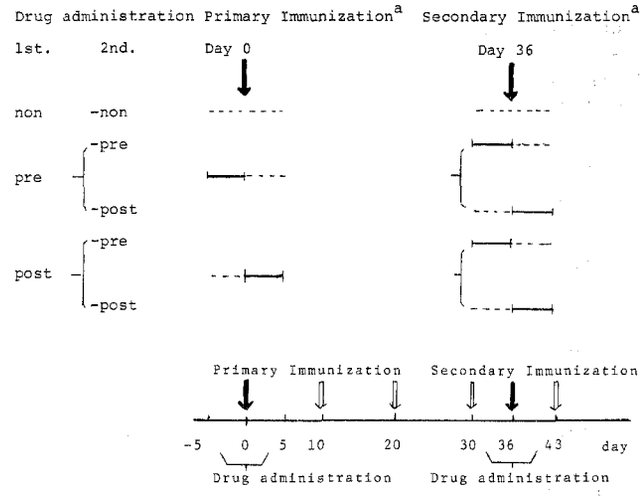
[a] Mice were immunized with 10 μg of DNP-As and 3 mg of $Al(OH)_3$ on days 0 and 36. Serum was collected from each mouse on days 10, 20, 30, and 43. Compound 13 was dissolved in a 2% $NaHCO_3$ solution and given 100 (mg/kg)/day po for 5 days before or after the primary and/or secondary immunization. Each value represents the mean ± SE of from 8 to 16 animals. * and ** = statistical significance from non-non group at $p < 0.05$ and $p < 0.01$, respectively. [b] Passive cutaneous anaphylaxis. Titers are shown in $n$ of the highest dilution ($2^n$).

Table IX. Effect of Compound 13 on Hemagglutinin Formation at Different Times during the Course of the Primary and/or Secondary Immunization in Female BALB/c Mice[a]

| drug administration | | PHA titer ($log_2$)[b] | | | |
|---|---|---|---|---|---|
| 1st | 2nd | day 10 | day 20 | day 30 | day 43 |
| non | -non | 4 | 6 | 6 | 10 |
| pre | -pre | 4.5 | 4 | 5 | 10 |
| | -post | | | | 9 |
| post | -pre | 4.5 | 3.5 | 5.5 | - |
| | -post | | | | 10 |

[a] Mice were immunized with 10 μg of DNP-As and 3 mg of $Al(OH)_3$ on days 0 and 36. Serum was collected from each mouse on days 10, 20, 30, and 43. Compound 13 was dissolved in a 2% $NaHCO_3$ solution and given 100 (mg/kg)/day po for 5 days before or after the primary and/or secondary immunization. Each value represents the titer of pooled sera of from 6 to 16 animals. [b] Passive hemagglutination. Titers are shown in $n$ of the highest dilution ($2^n$).

Chart II. Administration Schedule (2) of Compound 13




[a] Mice were immunized with 10 μg of DNP-As and 3 mg of $Al(OH)_3$ on days 0 (⇓) and 36 (⇓). Compound 13 was dissolved in a 2% $NaHCO_3$ solution and given 100 (mg/kg kg)/day po for 5 days before or after the primary and/or secondary immunization: ⟼ = drug administration for 5 days; non = nontreated, pre = preadministration; post = postadministration. Serum was collected from each mouse of days 10, 20, 30, and 43 (⇩) to estimate antibody titers.

21.0%; the amount of antigen employed in this experiment was expressed by the amount of protein. As adjuvants, killed *Bordetella pertussis*, $2 \times 10^{10}$/mL, supplied by the Kaken Pharmaceutical Co., and aluminum hydroxide gel [$Al(OH)_3$] were used for rats and mice, respectively. The aluminum hydroxide gel was prepared by the method of Ogita et al.[11] Briefly, a mixture of equal volumes of 2 N aluminum sulfate and 2 M sodium hydroxide was stirred for 10 min at room temperature; then the precipitate was crushed with a polytoron, suspended in distilled water, centrifuged, and washed until no sulfate ion could be detected. The suspension of aluminum hydroxide gel was adjusted to pH 8.0 with 0.02 M borate-buffered saline. According to a modification of the method of Tada and Okumura,[12] rats were immunized with 1 mg of DNP-As and $10^{10}$ killed *Bordetella pertussis*. Five days later, they were boosted with 0.5 mg of DNP–As alone to produce the IgE antibody, which is immunologically similar to the human IgE antibody. Mice were immunized ip with 10 μg of DNP-As and 3 mg of aluminum hydroxide gel. Test compounds were given po to the animals for 5 days before or after the immunization, and blood samples were collected in order to estimate the antibody titers.

**Evaluation of PCA.** The serum collected from each animal was diluted serially, and each serum (0.1 mL) was injected intradermally and in duplicate into the shaved back of normal male Wistar rats weighing 170–190 g; after 48 h in homologous PCA (or after 24 h in heterologous PCA), the animals were given an iv injection of 1 mL of saline containing 1 mg of DNP-As and 5 mg of Evans blue in their vein. After 30 min, the animals were sacrificed, and the PCA titer was measured. The PCA titer recorded is the greatest dilution that gave skin reactions with a diameter of greater than 5 mm.

**Evaluation of PHA.** This reaction was carried out by the method of Avrameas et al.[13] The serum obtained was serially diluted, and each portion of the serum (0.05 mL) was mixed with 0.05 mL of sheep red blood cells coupled with DNP-As including glutaraldehyde. After incubation for 2 h, at 37 °C, the agglutination at the bottom of the plate was evaluated. The PHA titer recorded is the greatest dilution that gave complete agglutination.

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(11) T. Ogita and H. Mizushima, *Igaku no Ayumi*, **100**, 812 (1977).
(12) T. Tada and K. Okumura, *J. Immunol.*, **106**, 1002 (1971).
(13) S. Avrameas, B. Taudou, and S. Chuilon, *Immunochemistry*, **6**, 67 (1969).