needles of N-(piperidinomethyl)benzamide, mp 128—129° (lit.,[4] mp 129—130°), which weighed 2.6 g (60%). IR spectra of both products were identical with those of the authentic specimens.

Reaction of NMB with Trifluoroacetic Acid——A solution of 5.4 g (0.02 mol) of NMB in 20 ml of trifluoroacetic acid was stirred at a room temperature for 2.5 hr. After evaporation of trifluoroacetic acid, N,N'-methylenebisbenzamide was deposited, filtered, and washed with petr. ether. Yield was 2.0 g (79%). Recrystallization from ethanol gave needles, mp 215—217° (lit.,[4] mp 218—219°). The combined filtrate was concentrated to afford an oil, which was distilled under reduced pressure to give 2.3 g (66%) of benzyl trifluoroacetate, bp 81—83° (40 mmHg) [lit.,[5] bp 178—179°]. IR spectra of both products were in agreement with those of the authentic specimens.

General Procedure for Reaction of NMB with Protic Materials in the Presence of Ferric Chloride——To a suspension of 3.2 g (0.02 mol) of powdered ferric chloride in 30 ml of THF 0.02 mol of the protic material (ethanol, acetic acid, phenol, thiophenol, and benzylmercaptan were used) was added. The mixture was refluxed for 1—4 hr. After removal of THF, 15 ml of water was added to the residue. Deposited N,N'-methylenebisbenzamide was filtered off and the filtrate was extracted with ether. The ethereal solution was dried over $MgSO_4$. After removal of ether, the residue was distilled under reduced pressure to give the product. Yields of the products are listed in Table I. Identities of the products were made by comparison of their IR spectra with those of the authentic specimens.

4) A. Einhorn, *Ann.*, **343**, 207 (1905).
5) U.T. Oliverio and E. Sawicki, *J. Org. Chem.*, **20**, 363 (1955).

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Studies on the Constituents of *Hedera rhombea* Bean. I.[1] Glycosides of Hederagenin

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Four triterpenoid saponins, tentatively named saponins K_3, K_6, K_{10} and K_{12}, were isolated from the stems of *Hedera rhombea* Bean (Araliaceae). They were identified with hederagenin glycosides represented as formulae I, III, V and IX, respectively.

Keywords——*Hedera rhombea* Bean; saponin; hederagenin glycosides; hederagenin 3-O-α-L-arabinopyranoside; hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; 3-O-α-L-arabinopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosylester; 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosylester

Hedera rhombea Bean (Kizuta in Japanese) is a evergreen viny plant of the family Araliaceae, which is widely distributed in Japan, Korea and China.

On the constituents of this plant, little has been known except for fatty acids[3] and a saponin which has not been elucidated.[4]

We have now investigated on the saponin constituents in the stems of this plant.

1) The 23th Annual Meeting of the Japanese Society of Pharmacognosy, Hiroshima, Nov. 1976.
2) Location; *a*) *Sugitani, Toyama*; *b*) *3 Ho, Kanagawa-machi, Kanazawa.*
3) G. Kurono and K. Sakai, *Kanazawa Daigaku Yakugaku Nempo*, **4**, 1 (1954).
4) S. Kuwata and T. Matsukawa, *Yakugaku Zasshi*, **54**, 13 (1934).

MeOH ext. from the stems of *Hedera rhombea* BEAN
- added H_2O
- H_2O soln.
- extd. with ether
  - ether layer
  - H_2O layer
    - extd. with AcOEt
      - AcOEt layer
        - dissolved in AcOEt–MeOH poured into Petr. benzine
          - ppt [A]
            - silica gel column chromatog. solv.: AcOEt saturated with H_2O
              - K_3, K_6
          - supernatant
      - H_2O layer
        - extd. with BuOH
          - BuOH layer
            - dissolved in MeOH poured into AcOEt
              - ppt [B]
                - silica gel column chromatog. solv.: $CHCl_3$–MeOH–H_2O (65:30:10. lower layer)
                  - K_{10}, K_{12}
          - H_2O layer

Chart 1

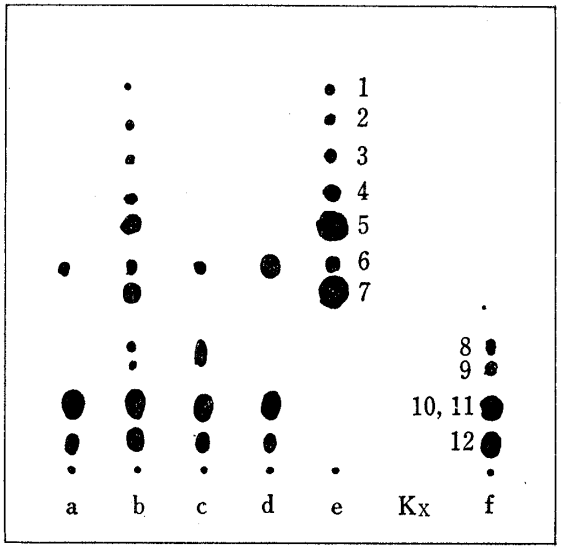

Fig. 1. TLC Patterns of MeOH Ext. from Each Part of *Hedera rhombea* BEAN, the Precipitates [A] and [B]

Plate: Kieselgel G.
Solv.: $CHCl_3$–MeOH–H_2O (65: 30: 10, lower layer).
a; leaves, b; stems, c; flowers, d; fruits, e; ppt[A] f; ppt[B].

The thin-layer chromatogram of the methanol extractives from the stems indicated the presence of twelve kinds of saponins, which were tentatively named saponins K_1—K_{12} in the order of increasing polarity. It was also suggested by thin-layer chromatography (TLC) that a part of these saponins were contained in the leaves, flowers and fruits (Fig. 1). Among them four kinds of saponins, K_3, K_6, K_{10} and K_{12} were isolated in pure state by fractionation procedure as shown in Chart 1 followed by repeated column chromatography. The present paper deals with the identification of these four saponins.

Saponin K_3 (I), mp 231—233° (dec.), $C_{35}H_{56}O_8 \cdot 3/2H_2O$, $[\alpha]_D$ +66.5° (pyridine), was acid hydrolyzed to give hederagenin and arabinose. On methanolysis its permethylate (II) gave 23-O-methyl hederagenin methylester and methyl 2,3,4-tri-O-methyl-L-arabinopyranoside. The nuclear magnetic resonance (NMR) spectrum of II shows anomeric proton signal at 4.16 ppm (doublet, J=6.0 Hz) whose coupling constant indicates that L-arabinose in I is α-linked to hederagenin.

Consequently saponin K_3 is hederagenin 3-O-α-L-arabinopyranoside (I).

Saponin K_6 (III), mp 248—249° (dec.), $C_{41}H_{66}O_{12} \cdot 2H_2O$, $[\alpha]_D$+18.8° (pyridine), consists of hederagenin, rhamnose and arabinose. The methanolysis of its permethylate (IV) yielded 23-O-methyl hederagenin methylester and methyl pyranosides of 2,3,4-tri-O-methyl-L-rhamnose and 3,4-di-O-methyl-L-arabinose. The mode of linkage of the L-arabinose unit

was regarded as α on the basis of the NMR spectrum of IV showing arabinosyl anomeric proton signal at 4.25 ppm as a doublet (J=6.5 Hz). The α-configulation of the L-rhamnose was suggested by the molecular rotation difference between I and III.

Therefore saponin K_6 is hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (III).

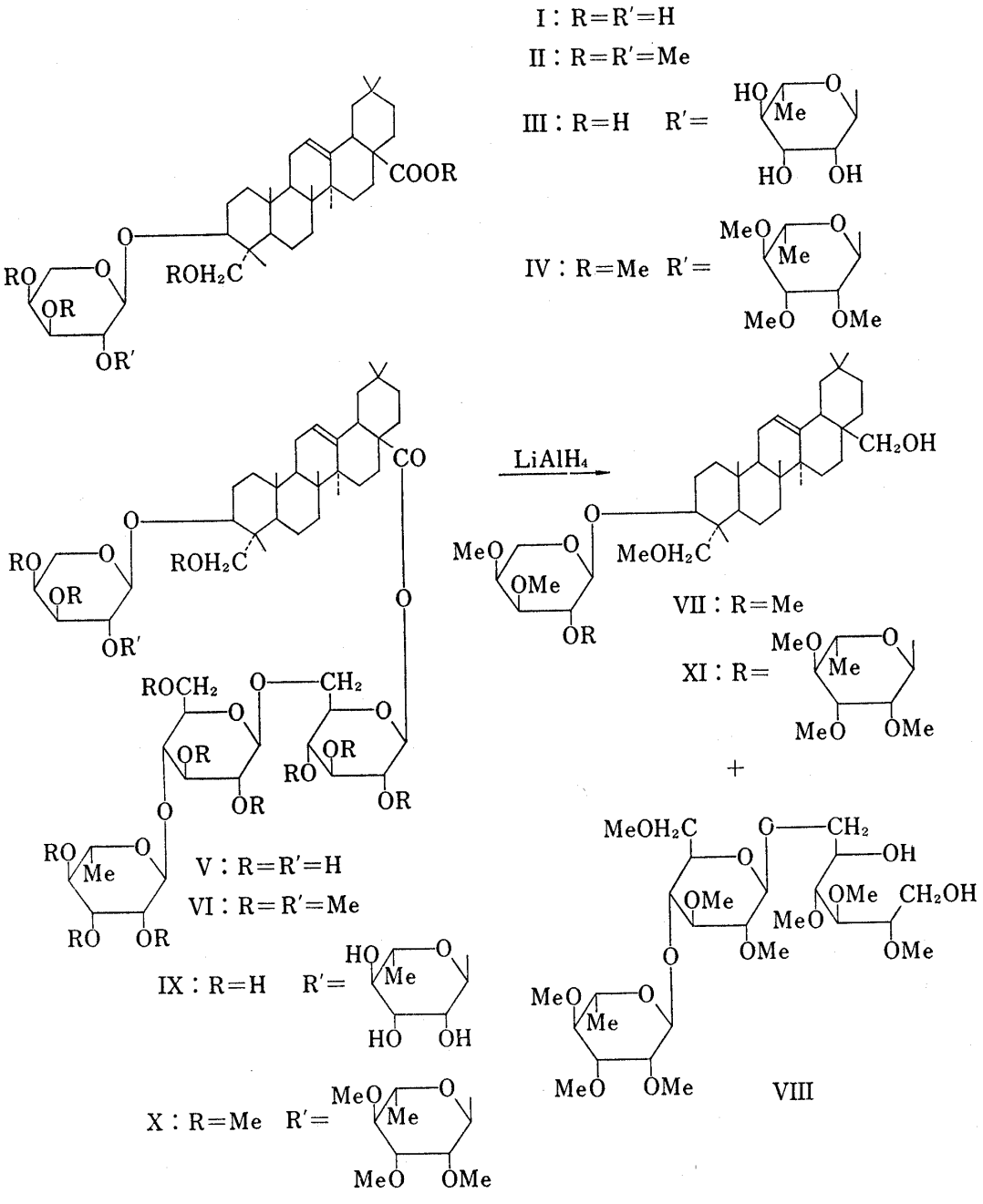

Chart 2

Saponin K_{10} (V), mp 211—213° (dec.), $C_{53}H_{86}O_{22}\cdot 2H_2O$, $[\alpha]_D$+10.2° (pyridine), is composed of hederagenin, rhamnose, arabinose and glucose, and gave I on alkaline hydrolysis. The methanolysis of its permethylate (VI) gave 23-O-methyl hederagenin and methyl pyranosides of 2,3,4-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose. Lithium aluminum hydride reduction of VI yielded a white powder (VII) and a colorless syrup (VIII). VII was proved to be the 28-ol correspond-

ing to II by direct comparisons with the synthetic sample. VIII gave on methanolysis a mixture of methyl pyranosides of 2,3,4-tri-O-methyl-L-rhamnose and 2,3,6-tri-O-methyl-D-glucose and a compound which could be considered to be 2,3,4-tri-O-methyl-D-sorbitol. On the basis of the above fact and the data obtained from the mass and NMR spectra, VIII was presumed to be 2,3,4-tri-O-methyl-α-L-rhamnopyranosyl-(1→4)-2,3,6-tri-O-methyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-methyl-D-sorbitol and identified by direct comparisons with an authentic sample. The mode of linkage of esterglycosyl glucose unit was regarded as β on the basis of the NMR spectrum of VI showing an anomeric proton signal of esterglycosyl glucose at 5.39 ppm as a doublet (J=7.8 Hz).

All the above results indicate that saponin K_{10} is represented as formula V.

Saponin K_{12} (IX), mp 222—226° (dec.), $C_{59}H_{96}O_{26}\cdot 3H_2O$, $[\alpha]_D$ −8.0° (pyridine), consists of hederagenin, rhamnose, arabinose and glucose, and gave III on alkaline hydrolysis. The methanolysis of its permethylate (X) yielded 23-O-methyl hederagenin and methyl pyranosides of 2,3,4-tri-O-methyl-L-rhamnose, 3,4-di-O-methyl-L-arabinose, 2,3,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose. Lithium aluminum hydride reduction of X afforded colorless needles (XI) and VIII. XI was proved to be the 28-ol corresponding to IV. The mode of linkage of esterglycosyl glucose residue was regarded as β on the basis of the NMR spectrum of X showing an anomeric proton signal of esterglycosyl glucose at 5.36 ppm as a doublet (J=8.4 Hz).

On the basis of these results saponin K_{12} is represented as formula IX.

These four saponins, I, III, V and IX reported here, were recently isolated from the pericarps of *Akebia quinata* DENCE.[5]

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra were taken at 100 MHz with a JEOL-JNM-MH-100 spectrometer in $CDCl_3$ solution and chemical shifts are given in δ (ppm) scale with tetramethylsilane as an internal standard. Infrared spectra were obtained with a JASCO-IRA-2 spectrometer. Optical rotations were measured with a JASCO-DIP-4 digital polarimeter using pyridine as solvent. Mass spectra were recorded on a JEOL-01SG-2 mass spectrometer. Gas liquid chromatography (GLC) was run on a Shimazu GC-6AM with flame ionization detecter using glass column (2 m×4 mmϕ) packed with 15% 1,4-butanediol succinate on chromosorb W (100—120 mesh).

Isolation of Saponins——The stems of *Hedera rhombea* BEAN collected in Toyama prefecture in May were extracted with hot MeOH. The MeOH extractives were treated as shown in Chart 1.

Saponin K_3——Colorless needles (from MeOH), mp 231—233° (dec.), $[\alpha]_D$ +66.5°. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. *Anal.* Calcd. for $C_{35}H_{56}O_8\cdot 3/2H_2O$: C, 66.53; H, 9.41. Found: C, 66.23; H, 9.31. I was heated with 1 N H_2SO_4 (dioxane–H_2O (1: 3)) for 3 hr and then diluted with water. The precipitates were recrystallized from MeOH to give colorless prisms, mp>300°, which were identified with hederagenin by direct comparisons. After being neutralized with $BaCO_3$, the filtrate was concentrated to small volume and examined by TLC and PPC to show the presence of arabinose.

Permethylate (II) of I——I (260 mg) was methylated by the Hakomori method.[6] The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and recrystallized from MeOH to give II (150 mg) as colorless needles, mp 182°. IR: no OH. MS m/e: 674, 175. NMR δ: 4.16 (1H, doublet, J=6.0 Hz). *Anal.* Calcd. for $C_{40}H_{66}O_8$: C, 71.18; H, 9.85. Found: C, 71.44; H, 10.04.

Methanolysis of II——II (30 mg) was boiled with 8% HCl–MeOH (3 ml) for 2 hr. The hydrolysate was neutralized with Ag_2CO_3, the precipitates were filtered off and the filtrate was evaporated. The residue was recrystallized from MeOH to give colorless needles, mp 187°, which were identified with 23-O-methyl hederagenin methylester by direct comparisons (TLC, IR, NMR). The methylated sugar in the mother liquor was identified with methyl 2,3,4-tri-O-methyl-L-arabinopyranoside by GLC.

Saponin K_6 (III)——Colorless needles (from MeOH), mp 248—249° (dec.), $[\alpha]_D$ +18.8°. IR ν_{max}^{KBr} cm^{-1}: 3400, 1700. *Anal.* Calcd. for $C_{41}H_{66}O_{12}\cdot 2H_2O$: C, 62.57; H, 8.97. Found: C, 62.85; H, 9.34. III was hydrolyzed in the same manner as I to give hederagenin, rhamnose and arabinose.

5) R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), 24, 1021 (1976).

Permethylate (IV) of III——III (1 g) was methylated and worked up as I to give V (780 mg) as colorless needles, mp 183—185°. IR: no OH. NMR δ: 4.25 (1H, doublet, J=6.5 Hz), 5.14 (1H, singlet). MS m/e: 349, 189. *Anal.* Calcd. for $C_{48}H_{80}O_{12}$: C, 67.89; H, 9.49. Found: C, 68.15; H, 9.68.

Methanolysis of IV——IV was methanolyzed and worked up as II to give 23-O-methyl hederagenin methylester and methyl pyranosides of 2,3,4-tri-O-methyl-L-rhamnose and 3,4-di-O-methyl-L-arabinose.

Saponin K_{10} (V)——A white powder (precipitated from MeOH–AcOEt), mp 211—213° (dec.), $[\alpha]_D$ +10.2°. IR ν_{max}^{KBr} cm^{-1}: 3400, 1730. *Anal.* Calcd. for $C_{53}H_{86}O_{22}\cdot 2H_2O$: C, 57.28; H, 8.16. Found: C, 57.18; H, 8.85.

Hydrolysis of V——i) With Acid: V was hydrolyzed as I to give hederagenin, rhamnose, arabinose and glucose.

ii) With Alkali: V was heated on water bath with 0.5 N KOH for 1 hr. The reaction mixture was diluted with water, neutralized with dil. HCl and extracted with AcOEt–BuOH (2: 1). The organic layer was washed with water and evaporated. The residue was recrystallized from MeOH to give colorless needles, mp 231—233° (dec.), which were identified with I by direct comparisons.

Permethylate (VI) of V——V (322 mg) was methylated by the Kuhn's method[7] (DMF 5 ml, Ag_2O 1.8 g, CH_3I 2 ml). The reaction mixture was added water, extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and passed through a silica gel column (eluent, benzene–acetone (9: 1)) to give VI (70 mg) as a white powder (precipitated from hexane), mp 117—122°. IR: no OH. MS m/e: 597, 189, 175. NMR δ: 4.99 (1H, singlet), 5.39 (1H, doublet, J=7.8 Hz). *Anal.* Calcd. for $C_{66}H_{112}O_{22}$: C, 63.03; H, 8.98. Found: C, 63.22; H, 9.04.

Methanolysis of VI——VI was methanolyzed and worked up as II to give an aglycone and methylated sugar portion. An aglycone, colorless needles (from MeOH), mp 216—217°, was identified with 23-O-methyl hederagenin by direct comparisons. Methylated sugars were identified by GLC as methyl pyranosides of 2,3,4-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose.

Reduction of VI——VI (60 mg) in tetrahydrofuran (6 ml) was treated with $LiAlH_4$ (80 mg). The reaction mixture was added water and extracted with ether and then with $CHCl_3$. The ether extract was evaporated and chromatographed over silica gel (eluent, benzene–acetone (97: 3)) to give VII as a white powder (precipitated from hexane), which was identified with the corresponding reduction product of II by direct comparisons (TLC, IR, NMR). The $CHCl_3$ extract was submitted to column chromatography of silica gel (eluent, benzene–aceton (4: 1)) to give a colorless syrup (VIII), $[\alpha]_D$ −40° ($CHCl_3$), NMR δ: 1.30 (3H, doublet, J=7.3 Hz), 4.33 (1H, doublet, J=7.8 Hz), 4.98 (1H, singlet). VIII was heated with 8% HCl–MeOH under reflux for 2 hr to yield a mixture of methyl pyranosides of 2,3,4-tri-O-methyl-L-rhamnose and 2,3,6-tri-O-methyl-D-glucose and a compound which could be considered to be 2,3,4-tri-O-methyl-D-sorbitol. VIII was identified with 2,3,4-tri-O-methyl-α-L-rhamnopyranosyl-(1→4)-2,3,6-tri-O-methyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-methyl-D-sorbitol by direct comparisons (IR, MS, NMR).

Saponin K_{12} (IX)——A white powder (precipitated from MeOH–AcOEt), m p222—226° (dec.), $[\alpha]_D$ −8.0°. IR ν_{max}^{KBr} cm^{-1}: 3400, 1730. *Anal.* Calcd. for $C_{59}H_{96}O_{26}\cdot 3H_2O$: C, 55.56; H, 8.26. Found: C, 55.25; H, 8.42.

Hydrolysis of IX——IX was hydrolyzed with acid as I to give hederagenin, rhamnose, arabinose and glucose, and with alkali as V to give III.

Permethylate (X) of IX——IX (2 g) was methylated (DMF 20 ml, Ag_2O 8 g, CH_3I 8 ml) and worked up as V to give a white powder (X) (500 mg) (precipitated from hexane), mp 120—122°. IR: no OH, MS m/e: 597, 349, 189. NMR δ: 5.00 (1H, singlet), 5.16 (1H, singlet), 5.36 (1H, doublet, J=8.4 Hz). *Anal.* Calcd. for $C_{74}H_{126}O_{26}$: C, 62.08; H, 8.87. Found: C, 62.90; H, 9.21.

Methanolysis of X——X was methanolyzed as II to yield 23-O-methyl hederagenin and methylated sugars which were identified by GLC as methyl pyranosides of 2,3,4-tri-O-methyl-L-rhamnose, 3,4-di-O-methyl-L-arabinose, 2,3,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose.

Reduction of X——X (380 mg) in tetrahydrofuran (30 ml) was treated with $LiAlH_4$ (340 mg) and worked up as VI to give XI as colorless needles (from MeOH) and VIII. XI was identified with corresponding reduction product of IV by direct comparisons (IR, TLC, NMR).

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6) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
7) R. Kuhn, *Angew. Chem.*, **67**, 32 (1955).