Na_2CO_3, and H_2O and then precipitated from DMF with ether; yield 0.82 g (49%), mp 214—216°, $[\alpha]_D^{25}$ −33.5° (c=0.9, DMF). Rf_1 0.63. Amino acid ratios in an acid hydrolysate: Glu 0.98, Phe 1.93, Gly 1.00, Leu 0.98, Met 0.80 (average recovery 81%). *Anal.* Calcd. for $C_{44}H_{55}N_7O_9S \cdot H_2O$: C, 60.32; H, 6.55; N, 11.19. Found: C, 60.32; H, 6.56; N, 11.18.

H-PCA-Phe-Phe-Gly-Leu-Met-NH_2——Z-PCA-Phe-Phe-Gly-Leu-Met-NH_2 (0.40 g) was treated with HF (approximately 5 ml) in the presence of anisole (0.5 ml) in an ice-bath for 30 min. The excess HF was removed by evaporation and the residue was dissolved in H_2O (5 ml), which was treated with Amberlite IR-4B (acetate form, approximately 3 g) for 30 min and then filtered. The filtrate was condensed *in vacuo* and the residue was treated with 5% NH_4OH. The resulting powder was washed with H_2O and recrystallized from MeOH; yield 0.21 g (62%), mp 225—230°, $[\alpha]_D^{26}$ −40.4° (c=0.3, DMF). Rf_1 0.67. Amino acid ratios in an acid hydrolysate: Glu 0.84, Phe 1.95, Gly 0.92, Leu 1.00, Met 0.86 (average recovery 83%). *Anal.* Calcd. for $C_{36}H_{49}N_7O_7S \cdot 1/2\ H_2O$: C, 58.99; H, 6.88; N, 13.37. Found: C, 58.98; H, 6.78; N, 12.77.

Z-PCA-Gln-Phe-Phe-Gly-Leu-Met-NH_2——To a solution of the TFA salt of H-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (prepared from 1.36 g of the Z(OMe)-derivative[4]) in DMF (20 ml), Et_3N (0.4 ml) and Z-PCA-ONP (0.60 g) were added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was treated with AcOEt. The resulting powder was washed batchwise as stated above and then precipitated from DMF with AcOEt; yield 1.17 g (79%), mp 230—232°, $[\alpha]_D^{26}$ −39.6° (c=0.8, DMF). Rf_1 0.52. Amino acid ratios in an acid hydrolysate: Glu 2.01, Phe 2.04, Gly 1,00. Leu 0.99, Met 0.75 (average recovery 84%). *Anal.* Calcd. for $C_{49}H_{63}N_9O_{11}S$: C, 59.67; H, 6.43; N, 12.78. Found: C, 59.40; H, 6.54; N, 12.58.

H-PCA-Gln-Phe-Phe-Gly-Leu-Met-NH_2——The above protected heptapeptide amide (0.40 g) was treated with HF (approximately 5 ml) in the presence of anisole (0.5 ml) and the product was isolated as stated above; yield 0.19 g (56%), mp 268—270°, $[\alpha]_D^{26}$ −51.7° (c=0.3, DMF). Rf_1 0.32. Amino acid ratios in an acid hydrolysate: Glu 1.87, Phe 1.96, Gly 1.00, Leu 1.00, Met 0.78 (average recovery 80%). *Anal.* Calcd. for $C_{41}H_{57}N_9O_9S \cdot H_2O$: C, 56.60; H, 6.84; N, 14.49. Found: C, 56.59; H, 6.78; N, 13.81.

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Studies on the Constituents of Bezoar. Characterization of Fatty Acids and Their Cholesteryl Esters

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The chemical investigation of Argentine bezoar showed the existence of cholesteryl esters of fatty acids (**1**), lithocholic acid, methyl cholate, methyl deoxycholate, methyl chenodeoxycholate, oleanolic acid, and ursolic acid besides the constituents already reported to be. The fatty acid compositions of **1**, as well as of free fatty acids (**2**) were scrutinized using gas chromatography and mass chromatography by converting them into the corresponding mixtures of methyl esters (**1e**) and (**2e**). These analyses showed that the mixtures consisted of C_{14} to C_{18} fatty acid esters with varing degree of relative abundance, palmitate and stearate being the major components in each of the mixtures.

Keywords——bezoar; fatty acid cholesteryl esters; fatty acids; bile acid methyl esters; oleanolic acid; ursolic acid; lithocholic acid; mass chromatography

The oriental drug "Bezoar", which is the dried gallstone of the ox, *Bos taurus* Linné var. *domesticus* Gmelin, has been used from ancient times as cardiotonics, antipyretics, analge-

1) Location: *1-1 Keyakidai, Sakado, Saitama.*

sics, antispasmodics, *etc.* and is also known as an ingredient of the home medicine "Rokushingan". There are many reports on its pharmacological properties,[2] but its constituents or the relationship between the constituent and the pharmacological effect of bezoar are not clarified enough. Up to now the following constituents have been reported;[3,4] bile pigments, cholesterol, fatty acids, bile acids and their conjugates, amino acids and peptides, lecithins, carotenoids, and inorganic substances. As the initial step to investigate the cardiotonic principle of bezoar, none of which is known, a comprehensive study was made on its constituents, whose result is reported in this paper.

The bezoar used in this work was that of Argentine, and it was extracted as shown in Chart 1.

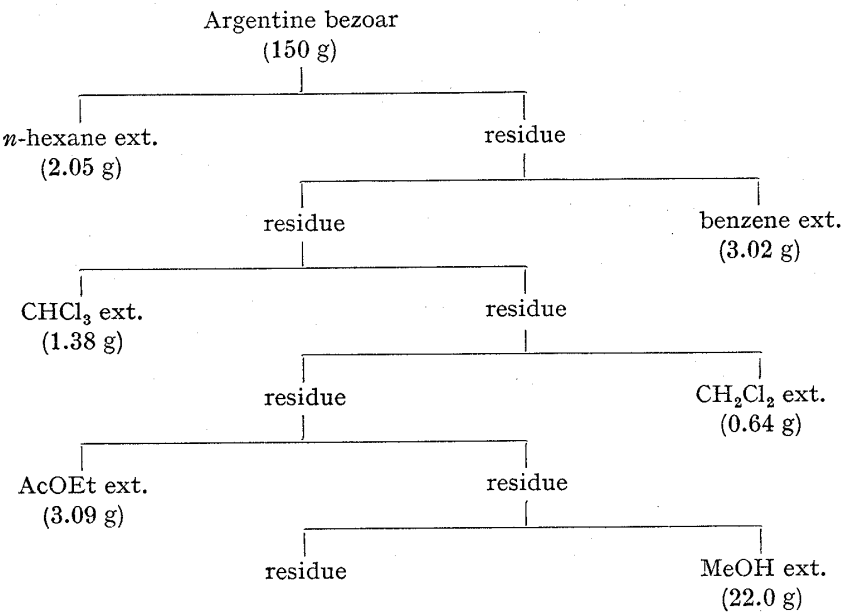

Chart 1

TABLE I. Identified Compounds in Each Extract of Bezoar

n-Hexane ext.	**1**,[a]	**2**,	**3**					
Benzene ext.	**1**,[a]	**2**,	**3**,	**4**,	**5**,	**6**,	**7**[b]	
$CHCl_3$ ext. / CH_2Cl_2 ext.	**2**,	**3**,	**4**,	**5**,	**6**,	**8**,	**9**,[b]	**10**[b]
AcOEt ext.	**4**,	**5**,	**6**,	**11**,[b]	**12**,[b]	**13**[b]		
MeOH ext.	**4**,	**5**,	**14**,	**15**,	**16**,	**17**		

(**1**) fatty acid cholesteryl esters, (**2**) free fatty acids, (**3**) cholesterol, (**4**) cholic acid, (**5**) deoxycholic acid, (**6**) chenodeoxycholic acid, (**7**) lithocholic acid, (**8**) bilirubin, (**9**) oleanolic acid, (**10**) ursolic acid, (**11**) methyl cholate, (**12**) methyl deoxycholate, (**13**) methyl chenodeoxycholate, (**14**) glycocholic acid, (**15**) taurocholic acid, (**16**) glycodeoxycholic acid, (**17**) taurodeoxycholic acid
a) These compounds were obtained in a comparable yield to that of free cholesterol.
b) These compounds were obtained in only minute amount.

2) S. Sugimoto, *Nippon Yakubutsugaku Zasshi*, **21**, 40 (1935) and its preceding papers; K. Hano, S. Hasegawa, K. Fujito, and T. Goto, *Nippon Yakurigaku Zasshi*, **40**, 43 (1944); R. Iwaki, M. Nakagawa, T. Kamemoto, Y. Hashiba, K. Ohashi, and S. Yoshikawa, *ibid.*, **60**, 529 (1964); R. Iwaki, T. Mori, and H. Nakagawa, *Yakugaku Zasshi*, **85**, 899 (1965); M. Kimura, E. Osada, and Y. Nogami, *ibid.*, **88**, 1367 (1968) and its preceding papers.
3) *a*) M. Nishimura and Y. Mori, *Tokyo Ijishinshi*, **63**, 177 (1939); *b*) D. Nakaoki and M. Yoshizaki, *Shoyakugaku Zasshi*, **9**, 11 (1955); *c*) S. Takahashi and T. Asakura, *Yakkyoku*, **8**, 982 (1957); *d*) M. Kimura, *Taisha*, **10**, 749 (1973) and the references cited therein.
4) Lithofellic acid is also reported as constituent of bezoar, but we think it is obtained from gastroliths, not from gallstones. R. van Tassel, *Bull. Soc. fr. Minéral. Cristallogr*, **95**, 106 (1972) and the references cited therein.

Each constituent was separated and purified using various chromatographic techniques, if necessary after converting them into suitable derivatives. The identified constituents of each extract are listed in Table I.

Among the compounds listed in Table I, compounds (**1**), (**11**), (**12**), and (**13**) have never been reported as constituents of bezoar. The latter three compounds were identified by comparison of their physical as well as spectral properties with those of the standard samples derived from the corresponding acids. In addition to these compounds, oleanolic acid (**9**) and ursolic acid (**10**), which are commonly found in plants, could be identified among many triterpenic compounds of quite minor amount.

Compounds (**1**) were separated from the extracts by column chromatography on silica gel eluted with carbon tetrachloride, and showed the ester absorption at 1720 cm^{-1} in the infrared spectrum and the peaks corresponding to cholesterol and fatty acids with 3α-proton at δ 4.7 in the nuclear magnetic resonance spectrum. Our attention was next paid to the examination of the fatty acid composition in **1** and **2**.[5]

As it was impossible to separate **1** and **2** into their components, they were transformed into mixtures of corresponding methyl esters (**1e**) and (**2e**), by trans-esterification for **1** and ethereal diazomethane for **2**. The gas chromatograms (GC) of **1e** and **2e** are reproduced in Fig. 1. By measuring the augmentation of peaks after co-injection with standard samples of methyl esters, peaks b to j could be estimated as those esters shown in the right half of Fig. 1.

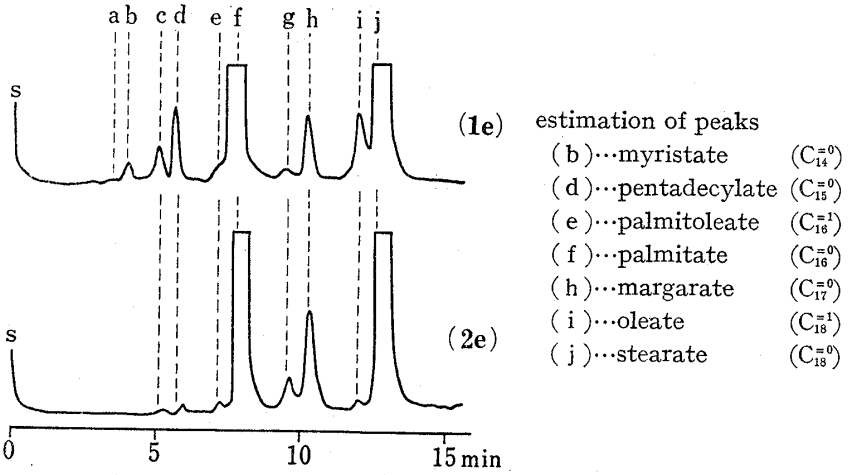

Fig. 1. Gas Chromatograms of the Mixtures **1e** and **2e**. (s=solvent)

In order to confirm the structures of the methyl esters unambiguously, the mass chromatograms (MC) of these mixtures were measured. As a representative example the MC of **1e** is shown in Fig. 2.

Based on these MCs, the mass spectrum of each peak was compared with that of the appropriate standard sample. The results of these analyses are summarized in Table II.[6] For those peaks denoted in Table II, good agreements were obtained.

Some comments should be made concerning this table. (A) It was concluded that peaks a, c, e, g, and i (of **2e**) were due to the branched-chain esters, for these peaks showed almost the same mass spectra as those of straight-chain isomers. But their mode of branching could not be made clear. (B) Peak **e** of both mixtures was at first assumed to be methyl palmitoleate (see Fig. 1), but its mass spectrum indicated that it was mainly due to the branched isomer

5) Though free fatty acids are known to be in bezoar, nothing is reported about their compositions.
6) Almost the same compositions of **1** and **2** were obtained when another 40 g of Argentine bezoar (purchased at different date) was examined, but **7**, **9**, **10**, **11**, **12**, and **13** could not be detected. This result may be due to the individual difference of bezoar. See ref. 3*b*.

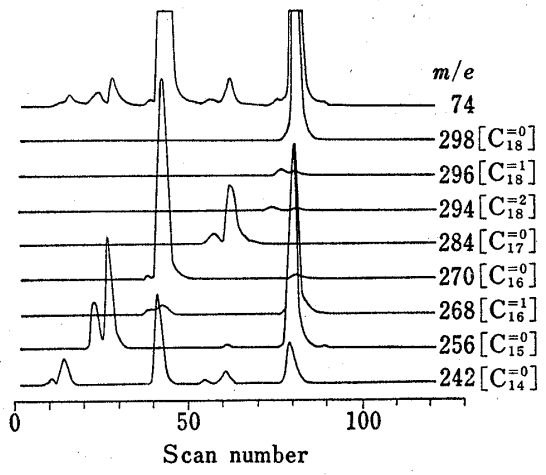

Fig. 2. The Mass Chromatogram of the Mixture (**1e**)

(The ion *m/e* 74 is characteristic of all the fatty acid methyl esters).

Table II. Composition of the Methyl Esters (**1e**) and (**2e**)

Parent fatty acid	Mixture (**1e**)	Mixture (**2e**)
Myristic acid[a] (n-$C_{14}^{=0}$)	b	×
Branched $C_{14}^{=0}$ acid	a	×
Pentadecylic acid[a] (n-$C_{15}^{=0}$)	d	d
Branched $C_{15}^{=0}$ acid	c	c
Palmitoleic acid (n-$C_{16}^{=1}$)	e	×
Palmitic acid[a,b] (n-$C_{16}^{=0}$)	f	f
Branched $C_{16}^{=0}$ acid	e	e
Margaric acid[a] (n-$C_{17}^{=0}$)	h	h
Branched $C_{17}^{=0}$ acid	g	g
Linoleic acid (n-$C_{18}^{=2}$)	i	×
Oleic acid[a] (n-$C_{18}^{=1}$)	i	i
Stearic acid[a,b] (n-$C_{18}^{=0}$)	j	j
Branched $C_{18}^{=0}$ acid	×	i

The alphabets a to j refer to the peaks in Fig. 1. ×; component not detected *a*) component identified by comparison with the standard sample *b*) major component. C_x^y means the fatty acid of x carbon atoms with number y of unsaturation in the chain. The type of designation is also applied to the methyl esters (ex. n-$C_{16}^{=0}$ ester=methyl palmitate).

of methyl palmitate. (C) Methyl esters of less than 13 or of more than 19 carbon atoms could be observed on GC in neither of mixtures. It is obvious from the foregoing result that MC is one of the most useful methods for the characterization of each component of a mixture without separation. The structures of other components are now under examination.

Experimental

Extraction of Argentine Bezoar——The pulverized Argentine bezoar (150 g) was extracted with n-hexane, benzene, AcOEt, and MeOH using an Asahina-type extraction apparatus. The $CHCl_3$ extract was obtained using a round-bottomed flask with magnetic stirring under N_2 current. The CH_2Cl_2 extract was obtained by heating the solvent under reflux. (The $CHCl_3$ and the CH_2Cl_2 extracts were almost identical in composition and they were combined.)

Trans-esterification of 1——The suspension of **1** (50 mg) in abs. MeOH (50 ml) containing a catalytic amount of MeONa was heated under reflux until disappearance of **1** on thin-layer chromatography. Usual work-up and chromatography of the product on silica gel furnished **1e** (17 mg) and **3** (23 mg).

Conditions for GC and GC-MS Analyses——A Shimadzu LKB-9000 with a GC-MS PAC-300 data processing system was used. These analyses were conducted under the following set of conditions. column; OV-1(1%) on Chromosorb W [80—100 mesh] in a 2 m×3 mm i.d. glass column, column temp.; initial at 140°, final at 300°, rate of heating 3°/min, accelerating voltage; 3.5 kV, $(m/e)_i=40$, $(m/e)_f=330$, interval; 8 sec, offset time; 1 min, stop time; 20 min.

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