Those tested were EDTA, sodium fluoride, potassium oxalate, and ammonium oxalate. Sodium citrate gave a larger value in the method, which might form acetone by the oxidizing agent, and hence it should not be used in this method.

The results of parallel tests with a 2,4-dinitrophenylhydrazine method[5] on blood are shown in Table Ⅲ. The individual value in both methods was in good agreement in an experimental error. The precision of this method was examined by carrying out 25 separate analysis on blood, and the standard deviation was 4.1% for a mean value of 2.89 mg./dl. of 3-hydroxybutyric acid as asetone. This method is accurate enough as a distillation method and may be acceptable for routine work.

Summary

A simple method has been presented for the determination of 3-hydroxybutyric acid in blood. It is based on the oxidation of 3-hydroxybutyric acid to acetone with potassium dichromate in sulfuric acid, which is distilled during the oxidation and determined by the previously established method with trinitrobenzene as a color developing agent.

Simple and compact oxidation-distillation equipment has been designed and successfully used, which made possible to analyze many samples at the same time.

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72. Masao Tomita, Toshiro Ibuka,*1 Yasuo Inubushi,*2 Yasuo Watanabe, and Matao Matsui*3 : Studies on the Alkaloids of Menispermaceuos Plants. CCX.*4 Alkaloids of *Stephania japonica* Miers.(Suppl. 9).*5 Structure of Hasubanonine and Homostephanoline.*6

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Hasubanonine, m.p. 116°, $C_{21}H_{27}O_5N$, was first isolated and named by Kondo, *et al.*[1] in 1951 from *Stephania japonica* Miers. The alkaloid contains four methoxyl groups, one N-methyl group and one conjugated carbonyl group. Hasubanonine gave hemipinic acid on permanganate oxidation and phenanthrene on zinc dust distillation.[1] Hofmann degradation of its methiodide afforded a methine base,[1,4] $C_{21}H_{27}O_5N$, which on heating with acetic anhydride generated 2-dimethylaminoethanol and acetylhasubanol (monoacetoxy-trimethoxyphenanthrene) (Ia). Hydrolysis of Ia followed by methylation gave

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*4 Part CCIX. M. Tomita, Y. Okamoto : Yakugaku Zasshi, 85, 456 (1965).
*5 (Suppl. 8). Y. Watanabe, H. Matsumura : *Ibid.*, 83, 991 (1963).
*6 A preliminary communication of this work appeared in Tetrahedron Letters, No. 40, 2937 (1964).
1) H. Kondo, M. Satomi, T. Odera : Ann. Rep. ITSUU Lab., 2, 35 (1951).
2) M. Satomi : *Ibid.*, 3, 37 (1952).

3,4,6,8-tetramethoxyphenanthrene (Ib).[1~3] Since formation of phenanthrene derivatives on acetolysis of the morphine-sinomenine alkaloids were characteristic of this type alkaloids, hasubanonine was considered to be a member of this group. On the basis above results, Kondo, *et al.* proposed the structure (IIa) for hasubanonine.[4]

Thereafter, the structure of ethylhasubanol was shown to be 6-ethoxy-3,4,8-trimethoxyphenanthrene (Ic) by direct comparison with a synthetic specimen by Watanabe, *et al.*[5] On the other hand, Bentley,[6] from biogenetic consideration of the alkaloid, has suggested the alternative structure (IIb) for hasubanonine.

In this paper, the complete structure of hasubanonine was shown to be represented by IIc.

The nuclear magnetic resonance (NMR) spectrum*[7] of hasubanonine (IIc), m.p. 116°, $C_{21}H_{27}O_5N$, confirmed the presence of four methoxyl groups (τ=5.92, 6.09, 6.20, and 6.36), one N-methyl group (τ=7.48), one active methylene group $\geqslant C\text{-}CH_2\text{-}C\overset{\nearrow}{\underset{\searrow}{}}O$ (τ=6.62, 1H, doublet, J=16 c.p.s. and τ=7.27, 1H, doublet, J=16 c.p.s.) and two aromatic hydrogens (τ=3.28, 2H, singlet). The infrared spectrum of IIc revealed the presence of a conjugated carbonyl group at 1664 cm^{-1} and an enolic double bond at 1600 cm^{-1}. The absence of olefinic hydrogen and the proton geminal to the methoxyl group in the NMR spectrum of the base indicates that the grouping $\geqslant C\text{-}CH_2\text{-}C(=O)\text{-}C(OCH_3)=C\overset{\nearrow}{\underset{\searrow}{}}OCH_3$ is present in the hasubanonine molecule (Fig. 1).

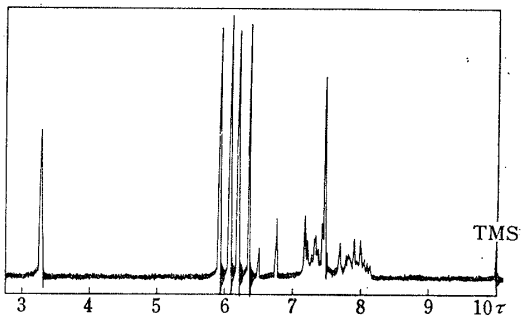

Fig. 1. Nuclear Magnetic Resonance Spectrum of Hasubanonine (IIc) (in $CDCl_3$)

The chemical shift was expressed as τ value (p.p.m.) referred to tetramethylsilane used as internal reference.

Just as reduction of sinomenine and methylsinomenine[7a,7b] with sodium borohydride gave two epimeric alcohols, reduction of hasubanonine (IIc) with the same reagent in aqueous methanol at room temperature provided two epimers, dihydrohasubanonine-A (IIIa) and dihydrohasubanonine-B (IIIb). Since oxidation of both IIIa and IIIb with chromium trioxide-pyridine complex regenerated the hasubanonine (IIc), it is apparent that IIIa must be an epimer of IIIb with respect to the configuration of hydroxyl group and that no transformation of the ring system of hasubanonine occurred during the above reduction process. On the other hand, oxidation of IIIa with activated manganese dioxide in chloroform afforded IIc, but of IIIb with the same reagent gave a lactam*[8] along with IIc.

Dihydrohasubanonine-A (IIIa) and -B (IIIb) revealed the following properties: IIIa, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3525 (hydroxyl), 1670 (enolic double bond), NMR 5.76 τ (1H, $\rangle C\underset{H}{\overset{OH}{<}}$, triplet), PPC*[9] Rf 0.68; IIIb, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (hydroxyl), 3300~3400 (hydrogen bonded

*[7] All NMR spectra were taken on Varian A-60 recording spectrometer in $CDCl_3$ with $SiMe_4$ as an internal standard.

*[8] The structure of this lactam will be stated in a separate paper.

*[9] PPC. Paper partition chromatography, Toyo Filter Paper No. 50, solvent AcOH-BtOH-H_2O=10:63:27. Spots of the bases were detected with Dragendorff reagent.

3) H. Kondo, M. Satomi, T. Odera: Ann. Rep. ITSUU Lab., 4, 45 (1953).
4) H. Kondo, M. Satomi: *Ibid.*, 8, 41 (1957).
5) Y. Watanabe, H. Matsumura: Yakugaku Zasshi, 83, 991 (1963).
6) K. W. Bentley: Experientia, 12, 251 (1956).
7) *a*) K. Okabe: Yakugaku Zasshi, 82, 1496 (1962). *b*) *Idem*: *Ibid.*, 82, 1503 (1962).

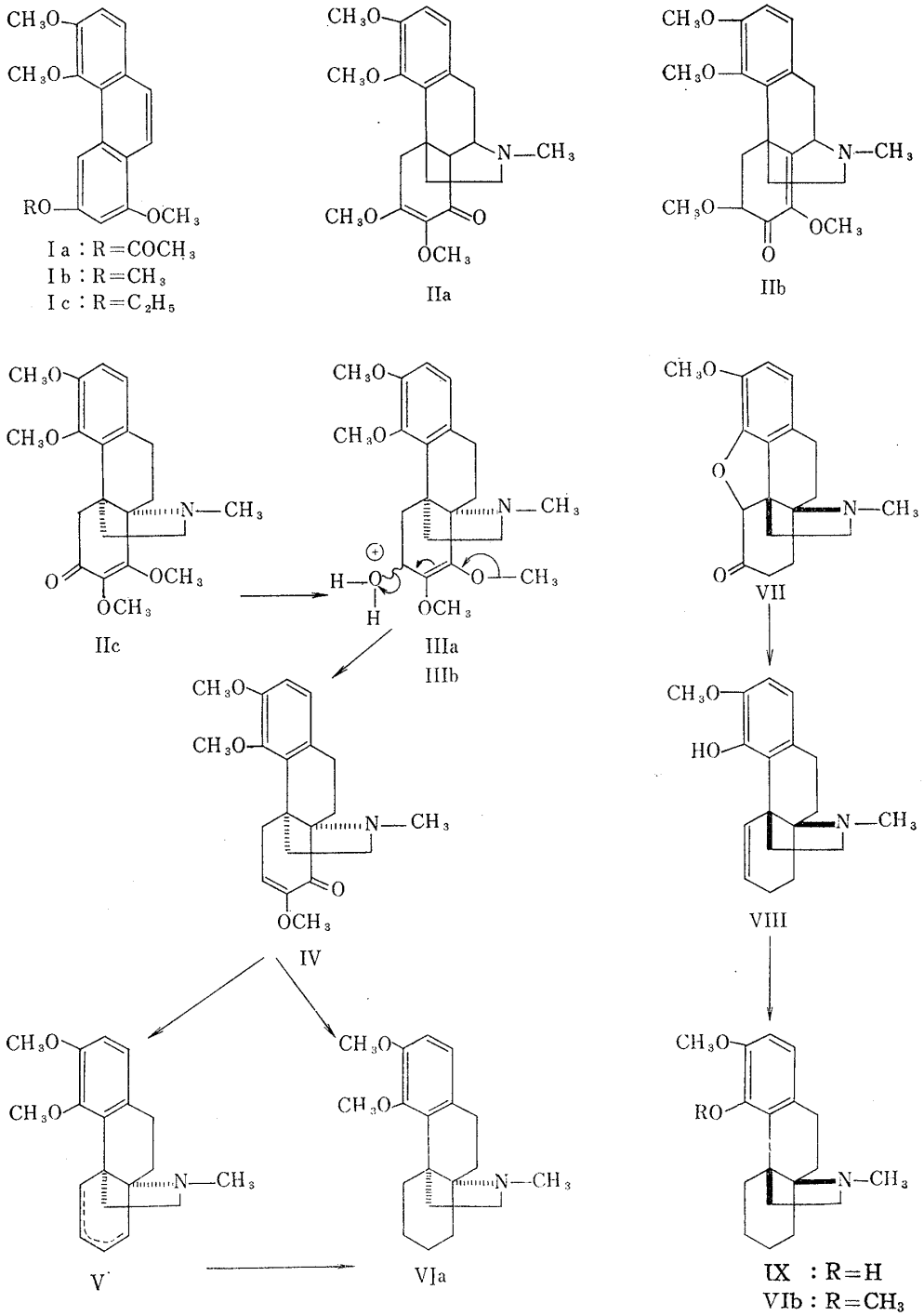

Chart 1.

hydroxyl), 1670 (enolic double bond), NMR 6.12 τ $\left(1H, >C<\underset{H}{OH}\right)$, PPC Rf 0.67. IIIb was more strongly adsorbed on a chromatographic alumina column than its epimer IIIa.[8] In the NMR spectrum of IIIb the signal of the proton geminal to the hydroxyl group appeared at higher field than its epimer IIIa.[9] In the PPC IIIb revealed a lower Rf

8) *a*) D. H. R. Barton: Experientia, **6**, 316 (1950); *Idem*: J. Chem. Soc., **1953**, 1027; D. H. R. Barton, R. C. Cookson: Quart. Rev. Chem. Soc., **10**, 44 (1956). *b*) D. Elad, D. Ginsburg: J. Chem. Soc., **1954**, 302.

9) *a*) R. U. Lemieux, R. K. Kulling, H. J. Bernstein, W. G. Schneider: J. Am. Chem. Soc., **80**, 6098 (1958). *b*) J. N. Shoolery, M. T. Rogers: *Ibid.*, **80**, 5121 (1958).

value than that of its epimer IIIa.[10] All these findings indicate that the hydroxyl group of IIIa has the *quasi*-axial conformation and that of IIIb has *quasi*-equatorial conformation.

Both IIIa and IIIb on mild treatment with dil. hydrobromic acid caused demethanolization producing a conjugated carbonyl compound (IV). IV was characterized as its hydrobromide, m.p. 232° (decomp.), $C_{20}H_{25}O_4N \cdot HBr$, IR ν_{max}^{Nujol} cm^{-1} : 1680 (conjugated carbonyl), 1629 (enolic double bond) and 2200~2700 ($\overset{+}{N}$-H). In the infrared spectrum of free base (IV), a conjugated carbonyl band at 1671 cm^{-1} and an enolic double bond absorption band at 1646 cm^{-1} were observed. In the NMR spectrum, IV revealed three methoxyl group (6.10 (3H), 6.18 (3H) and 6.43 τ (3H)) and one olefinic hydrogen (4.33 τ (1H), triplet). Therefore, the presence of the grouping $>\overset{|}{C}-CH_2-\underset{H}{\overset{|}{C}}=\underset{OCH_3}{\overset{|}{C}}-\overset{\diagup}{\underset{\diagdown}{C}}\overset{}{=}O$ in the conjugated carbonyl compound (IV) was established.

Clemmensen reduction of IV afforded an olefinic (V) and a saturated compound (VIa) and this reduction sequence, IV→V and VIa has analogy in the reduction of sinomenine and its derivatives with the same reagent.[11] The olefinic compound (V), m.p. 103°, $C_{19}H_{25}O_2N$, showed two methoxyl groups (6.15 (3H) and 6.21 τ (3H)) and two olefinic protons (4.10 (1H, sextet) and 4.50 τ (1H, sextet)) in the NMR spectrum. Catalytic hydrogenation of V over platinum oxide in acetic acid gave the saturated compound (VIa) which was characterized as its hydrobromide, m.p. 270~271° (decomp.), $C_{19}H_{27}O_2N \cdot HBr$, $[\alpha]_D$ +33° (MeOH).

The validity of the structure of VIa was verified by comparison of VIa with an authentic sample of VIb derived from dihydroindolinocodeinone (VII)[12] (VIa is the enantiomer of VIb).

Huang-Minlon reduction[13] of the ketone (VII) afforded deoxodihydroindolinocodeinone (VIII) as colorless oil which was characterized as its oxalate, m.p. 251°(decomp.), $C_{18}H_{23}O_2N \cdot (CO_2H)_2$. VIII like sinomenine gives rise to characteristic strong blue color when treated with 2,6-dichloroquinone-4-chloroimide and this test suggested the presence of the phenolic C_4-hydroxyl group. Catalytic hydrogenation of VIII over platinic oxide gave deoxotetrahydroindolinocodeinone (IX), characterized as its hydrochloride, m.p. 263~264°. Methylation of IX with Rodionov reagent[14] in boiling toluene afforded a compound (VIb) characterized as its hydrobromide, m.p. 270~271° (decomp.), $C_{19}H_{27}O_2N \cdot HBr$, $[\alpha]_D$ −42° (MeOH).

As shown in Table I, properties of VIa derived from hasubanonine (IIc) were quite identical with those of VIb derived from dihydroindolinocodeinone (VII) except the sign of rotation.

Rüll[15] and Tsuda, *et al.*[12b,16] have pointed out that the signal attributable to the C_9-H of the compounds possesing the skeleton of type (X) appeared at 6.35~6.95 τ in their NMR spectra, while in the derivatives of indolinocodeine (XI) series no signal

10) K. Savard : J. Biol. Chem., **202**, 457 (1953).
11) H. Kondo, E. Ochiai : Ann., **470**, 224 (1929); *Idem* : Yakugaku Zasshi, **44**, 8 (1924); K. Okabe : Ann. Repts. Shionogi Research Lab., **11**, 49 (1961); Y. K. Sawa, N. Tsuji, S. Maeda : Tetrahedron, **15**, 144 (1961).
12) *a*) S. Okuda, K. Tsuda, S. Yamaguchi : J. Org. Chem., **27**, 4121 (1962). *b*) *Idem* : "The 7th Symposium on the Chemistry of Natural Products, Japan" (Fukuoka, Oct. 1963), Symposium Abstracts p. 72 (1963).
13) T. D. Perrine, L. F. Small : J. Org. Chem., **17**, 1540 (1952); K. Goto, I. Yamamoto : Proc. Japan Acad., **36**, 145 (1960); I. Seki : Ann. Rept. Takamine Lab., **13**, 67 (1961).
14) W. Rodionov : Bull. soc. chim. France, **39**, 305 (1926).
15) T. Rüll : *Ibid.*, **1963**, (3), 586.
16) S. Okuda, S. Yamaguchi, K. Tsuda : This Bulletin, **12**, 104 (1964); *Idem* : *Ibid.*, **11**, 1466 (1963).

Table I.

		VIa	VIb
Free base	IR ($CHCl_3$)	identical	
	NMR ($CDCl_3$)		
	thin-layer chromatography		
Hydrobromide	formulae	$C_{19}H_{27}O_2N \cdot HBr$	$C_{19}H_{27}O_2N \cdot HBr$
	appearance	colorless prisms	colorless prisms
	m.p.	270~271° (decomp.)	270~271° (decomp.)
	$[\alpha]_D$: (MeOH)	+33°	−42°
	IR (Nujol)	identical	

attributable to C_9-H was appeared in this region.[12b] In accordance with these observations, all derivatives of hasubanonine showed no signal attritutable to this proton in this region of their NMR spectra.

On the basis of above results, the structure of hasubanonine is unambiguously assigned to the formula (IIc) including the absolute stereostructure. Hasubanonine is not morphine or sinomenine type alkaloids but a novel skeletal alkaloid, hitherto not known in the natural sources. Then, authors would like to propose the name "hasubanan"*10 for the skeleton (XII). In this skeleton, the numbering depends on the same manner with that of morphinan and the asymmetric centers at C-13 and C-14 should be *R* and *S*, respectively.

Homostephanoline was first isolated from *Stephania japonica* Miers. by Kondo, *et al.*[17] in 1928. Thereafter, Tomita, *et al.*[18] have confirmed the empirical formula to be $C_{20}H_{25}O_5N=C_{16}H_{12}O \cdot (OH) \cdot (N\text{-}CH_3) \cdot (OCH_3)_3$. On methylation of homostephanoline with diazomethane afforded hasubanonine,[18] so that the hydroxyl group must be a phenolic or enolic one.

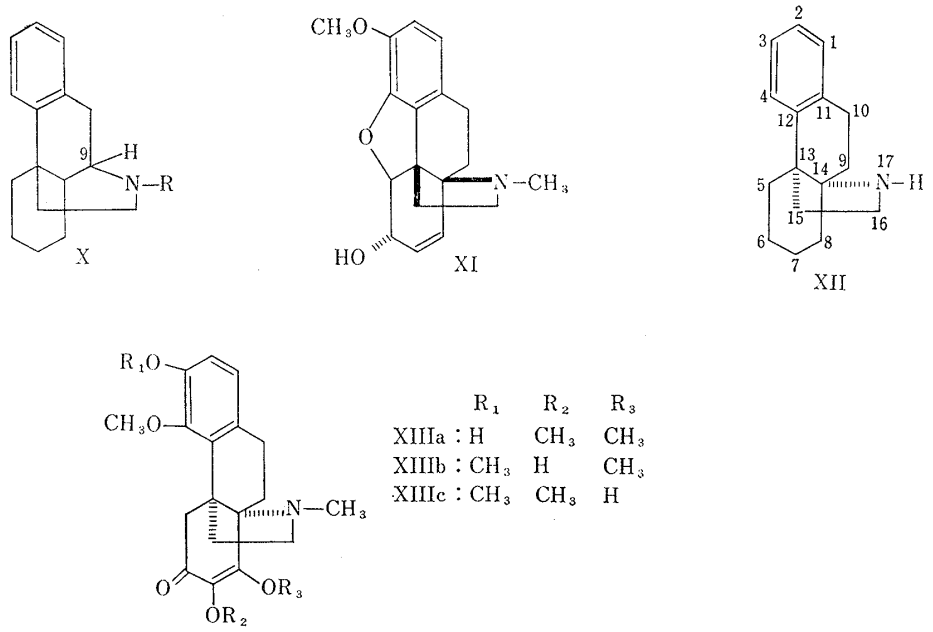

Chart 2.

*10 The authors are grateful to Prof. K. Tsuda and Dr. S. Okuda, Tokyo University, for their generous offer in consenting to designate the skeleton XII as hasubanan.

17) H. Kondo, T. Sanada : Yakugaku Zasshi, **48**, 1141 (1928).

18) M. Tomita, Y. Watanabe : *Ibid.*, **76**, 856 (1956).

Homostephanoline showed the conjugated carbonyl band at 1665 cm^{-1} and the band due to an enolic double bond at 1603 cm^{-1} in its infrared spectrum and three methoxyl groups at 5.92, 6.14, and 6.40 τ in its nuclear magnetic resonance spectrum. Negative 2,6-dichloroquinone-4-chloroimide test of homostephanoline eliminates the possibility of the hydroxyl group at C-4 position.

Therefore, on the structure of homostephanoline the reasonable conclusion being drawn from available data is that the structure of homostephanoline must be represented by XIIIa, XIIIb, or XIIIc. Homostephanoline is the second example of the alkaloid possessing the hasubanan skeleton.

Experimental*11

Dihydrohasubanonine-A (IIIa) and Dihydrohasubanonine-B (IIIb)——To a solution of 64 mg. of hasubanonine (IIc) in 5 ml. of 10% aq. MeOH was added 21 mg. of sodium borohydride with stirring for 4 hr., and then the excess sodium borohydride was decomposed with 1% AcOH. The solvent was evaporated under reduced pressure to dryness and 10 ml. of 3% aq. NH_4OH was added and extracted with ether. The ether solution, after washed and dried over Na_2SO_4, was evaporated to give 62 mg. of colorless oil. The oil was dissolved in minimum amount (1 ml.) of benzene, and was chromatographed over alumina column and elution with benzene gave 25 mg. of dihydrohasubanonine-A (IIIa) and then with benzene-EtOH (99:1) gave 36 mg. of dihydrohasubanonine-B (IIIb).

Oxidation of Dihydrohasubanonine-A (IIIa). a) Oxidation of IIIa with Chromium Trioxide-Pyridine Complex——A solution of 75 mg. of dihydrohasubanonine-A (IIIa) in 2 ml. of pyridine was treated with chromium trioxide pyridine complex (CrO_3 120 mg., pyridine 10 ml.) with stirring for 5 hr. The reaction mixture was poured into ice water and extracted with ether. The ether extract was washed, dried over Na_2SO_4, and evaporated giving brown oil, which was chromatographed over alumina column (1×5 cm.) from benzene and eluted with the same solvent. Recrystallization of the eluate from MeOH gave 5 mg. of crystals, m.p. 115°. The product was proved to be identical with an authentic sample of hasubanonine by comparison of IR spectra ($CHCl_3$) and mixed melting point determination.

b) Oxidation of IIIa with Manganese Dioxide——A solution of IIIa (49 mg.) in $CHCl_3$ (10 ml.) was treated with activated MnO_2 (500 mg.) with stirring at room temperature for 4 hr. and worked up as usual. The product, m.p. 115° (from MeOH) was identified with the authentic sample of hasubanonine by comparison of IR spectra and mixed melting point determination. Yield 40 mg.

Oxidation of Dihydrohasubanonine-B (IIIb). a) Oxidation of IIIb with Chromium Trioxide-Pyridine Complex——A solution of 66 mg. of dihydrohasubanonine-B (IIIb) in 2 ml. of pyridine was treated with CrO_3-pyridine complex (CrO_3 120 mg., pyridine 10 ml.) at 0° with stirring for 5 hr. After worked up by the same procedure as for oxidation of IIIa, the product was recrystallized from MeOH to give 3 mg. of crystals (IIc), m.p. 115°. On admixture of the product with an authentic sample of hasubanonine, no depression of melting point was observed and the IR spectra of two compounds were identical.

b) Oxidation of IIIb with Manganese Dioxide——A solution of IIIb (165 mg.) in $CHCl_3$ (10 ml.) was treated with MnO_2 (1.65 g.) with stirring at room temperature for 11 hr. After filtration, the solvent was removed under reduced pressure and the residual oil (160 mg.) was dissolved in ether (50 ml.). The ether solution was extracted with 3% aq. tartaric acid (20 ml.×4): the ether layer was washed, dried over Na_2SO_4, evaporated and recrystallization from ether afforded 81 mg. of a lactam,*8 m.p. 152~156°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1688 (five membered lactam). The aq. tartaric acid solution separated from the ether layer was then basified with aq. NH_4OH and extracted with ether, and then the ether solution was washed, dried over Na_2SO_4 and evaporated to give 65 mg. of hasubanonine (IIc), m.p. 114~116° (from MeOH).

Treatment of Dihydrohasubanonine-A (IIIa) with dil. Hydrobromic Acid——A solution of IIIa (10 mg.) in acetone (1 ml.) was heated with dil. hydrobromic acid (0.5 ml. of 1% HBr) for 5 min. at 60°. After cooling, the resulting crystalline hydrobromide was recrystallized from acetone to give the conjugated carbonyl compound (IV) hydrobromide as colorless prisms, m.p. 232° (decomp.). *Anal.* Calcd. for $C_{20}H_{25}O_4N \cdot HBr$: C, 56.61; H, 6.18. Found: C, 56.58; H, 6.27. IR ν_{max}^{Nujol} cm^{-1}: 2200~2700 ($\overset{+}{N}$-H), 1680 (conj. C=O), 1629 (enolic C=C).

The above hydrobromide was dissolved in H_2O and made alkaline with dil. NH_4OH, and extracted with ether. The ether extract was washed, dried over Na_2SO_4 and evaporated to dryness. The IR spectrum (in $CHCl_3$) of the amorphous free base (IV) thus obtained showed the band at 1671(conj. C=O) and at 1646 cm^{-1} (enolic C=C).

*11 All melting points are uncorrected and determined with Yanagimoto Micro Melting Point Apparatus.

Treatment of Dihydrohasubanonine-B (IIIb) with dil. Hydrobromic Acid——To a solution of 19 mg. of IIIb in acetone (1 ml.) was gradually added to 1% hydrobromic acid (0.1 ml.), and then the solution was heated at 60° for 5 min. After cooling, colorless crystals separated. The crude crystals were recrystallized from MeOH to give 18 mg. of IV hydrobromide as colorless prisms, m.p. 232° (decomp.). *Anal.* Calcd. for $C_{20}H_{25}O_4N \cdot HBr$: C, 56.61; H, 6.18. Found: C, 56.44; H, 6.21.

The IR spectrum (in Nujol) of IV hydrobromide derived from IIIb was superimposable with that of the hydrobromide derived from IIIa.

Clemmensen Reduction of the Conjugated Carbonyl Compound (IV)——IV (450 mg.) was dissolved in conc. HCl (4 ml.), and amalgamated zinc prepared from 3 g. of mossy zinc, 300 mg. of mercuric chloride and 5 ml. of 3% hydrochloric acid was added in portions over a period of 2 hr.

After the addition has completed the mixture was heated at 70° for 6 hr., during which time 9 ml. of conc. HCl was added in portions. After standing overnight, the acidic aqueous layer is decanted, and after made alkaline with aq. NH_4OH, extracted with ether. The ether extract was washed, dried over Na_2SO_4 and evaporated to give colorless oil (330 mg.) which is purified by repeated chromatography over alumina column from benzene. Continued elution with benzene gave two compounds; an olefinic (V) (30 mg.) and a saturated compound (VIa) (150 mg.). The saturated compound (VIa), which forms colorless crystals resisting to recrystallization, was quite identical with VIb derived from dihydroindolinocodeinone (VII); a) thin-layer chromatography (Al_2O_3–$CHCl_3$ system, Kieselgel G–MeOH system), b) IR spectra (in $CHCl_3$) and c) NMR spectra (in $CDCl_3$). VIa was characterized as its hydrobromide: VIa hydrobromide, m.p. 270~271° (decomp.) (acetone), colorless prisms. $[\alpha]_D^{31}$ +33° (c=1.00, MeOH). *Anal.* Calcd. for $C_{19}H_{27}O_2N \cdot HBr$: C, 59.69; H, 7.38. Found: C, 59.90; H, 7.31.

The IR spectra (in Nujol) of VIa hydrobromide and VIb hydrobromide were quite superimposable. The olefinic compound (V) showed m.p. 103° (hexane-ether=10:1); colorless prisms; $[\alpha]_D^{30}$ −140° (c=0.50, $CHCl_3$). *Anal.* Calcd. for $C_{19}H_{25}O_2N$: C, 76.22; H, 8.42. Found: C, 76.37; H, 8.59.

Catalytic Hydrogenation of the Olefinic Compound (V)——A solution of V (9 mg.) in 10% AcOH (5 ml.) was hydrogenated over PtO_2 for 4 hr. at room temperature. The catalyst was filtered off, washed with 3% AcOH and the combined filtrates were made alkaline with dil. NH_4OH and extracted with ether. The ether extract was washed, dried and evaporated to give colorless oil VIa. VIa was characterized as its hydrobromide, colorless prisms, m.p. 270~271° (decomp.) (acetone). *Anal.* Calcd. for $C_{19}H_{27}O_2N \cdot HBr$: C, 59.69; H, 7.38. Found: C, 59.96; H, 7.29.

The IR spectra (in Nujol) of the VIa hydrobromide and VIb hydrobromide were quite identical.

Huang-Minlon Reduction of Dihydroindolinocodeinone (VII)——A mixture of VII (40 mg.), ethylene glycol (2 ml.) and 85% hydrazine hydrate (1 ml.) was heated at 100° for 1 hr., and after cooling KOH pellets (500 mg.) was added and the reaction mixture was again heated for 1 hr. at 130°. After cooling, the reaction mixture was poured into ice water (20 ml.) and made alkaline with NH_4Cl and then extracted with ether. The ether extract was washed, dried over Na_2SO_4 and evaporated. The colorless residue (30 mg.) was chromatographed over alumina column from benzene and elution with benzene gave deoxodihydroindolinocodeinone (VIII) (15 mg.) as colorless oil which resisted to crystallization. VIII was characterized as its oxalate; m.p. 251° (decomp. with efferv.). *Anal.* Calcd. for $C_{18}H_{23}O_2N \cdot (CO_2H)_2$: C, 63.98; H, 6.71; N, 3.73. Found: C, 63.69; H, 6.81; N, 3.60.

The free base VIII showed a single spot on thin-layer chromatography and strong blue color when treated with 2,6-dichloroquinone-4-chloroimide.

Catalytic Hydrogenation of VIII——A solution of VIII (0.25 g.) in MeOH (8 ml.) was hydrogenated over PtO_2 (20 mg.) for 3 hr. at room temperature. The catalyst was filtered off, washed with MeOH and the combined filtrates were evaporated to dryness under reduced pressure. The residue was dissolved in 3% HCl and then made alkaline with aq. NH_4OH and extracted with ether. The ether extract was washed, dried and evaporated to give 230 mg. of colorless residue of deoxotetrahydroindolinocodeinone (IX) which was resisted to crystallization. Because of its unsatisfactory nature for characterization IX was derived to its hydrochloride hemihydrate; colorless prisms, m.p. 263~264° (decomp.). *Anal.* Calcd. for $C_{18}H_{25}O_2N \cdot HCl \cdot 1/2\,H_2O$: C, 64.96; H, 8.18; N, 4.21. Found: C, 65.25, 65.23; H, 8.33, 8.49; N, 4.41.

Methylation of IX with Rodionov Reagent——IX (120 mg.) was dissolved in anhyd. toluene (15 ml.), and then Rodionov reagent (2 ml. of MeOH solution) was added. The mixture was heated at 90° for 1 hr. to remove MeOH, and then at 130° with stirring for 8 hr. After cooling H_2O (10 ml.) was added and extracted with ether. The extract was washed, dried over Na_2SO_4. Evaporation of the solvent gave colorless oil (VIb, 101 mg.). VIb hydrobromide; colorless prisms, m.p. 270~271° (decomp). $[\alpha]_D^{23}$ −42° (c=0.833, MeOH). *Anal.* Calcd. for $C_{19}H_{27}O_2N \cdot HBr$: C, 59.69; H, 7.38; N, 3.67. Found: C, 59.64; H, 7.51; N, 3.87.

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Summary

The structure of hasubanonine was reexamined and the complate constitution (IIc) including the absolute stereostructure was presented. The structure of homostephanoline was also investigated and three possible structure for this alkaloid were shown in formulae (XIIIa), (XIIIb), and (XIIIc). These two alkaloids have a novel skeleton (XII) which has not been known hitherto in the natural sources, for which the name "hasubanan" was proposed.

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73. Tameto Okanishi, Akira Akahori, and Fumio Yasuda : Studies on the Steroidal Components of Domestic Plants. XLVII.*1 Constituents of the Stem of *Smilax sieboldi* Miq. (1). The Structure of Laxogenin.

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Smilax sieboldi Miq. is a climbing shrub of the Liliaceae family native to Japan, Korea, and China. It is well known that the various species of Smilax, native to Middle and South America, are the better plant source of smilagenin or sarsasapogenin, but the steroidal constituents of the oriental plants belonging to the same genus have not yet been reported, except diosgenin from *S. china* L.[1] Recently we obtained a new steroidal sapogenin together with tigogenin (25D,5α-spirostan-3β-ol) and neotigogenin (25L,5α-spirostan-3β-ol) from the stem of this plant.

The physical constants of the new sapogenin are as follows : $C_{27}H_{42}O_4$; m.p. 210~212°; $[\alpha]_D^{24.5}$ −86.3°; IR ν_{max}^{Nujol} cm^{-1} : 3490 (-OH), 1713 (>C=O), 982, 920, 901, 868, 920<901 (F-ring). Acetylation of this new sapogenin with acetic anhydride and pyridine afforded a monoacetate (Ib), $C_{29}H_{44}O_5$, m.p. 219~222°, $[\alpha]_D^{25}$ −88.6°, and its infrared absorption spectra showed an acetyl band but not a hydroxyl band. Thus, this sapogenin was considered to be a 25D-monohydroxysapogenin having a six-membered ketone group. Although the known monohydroxy-monoketo-spirostanes are hecogenin (3β-hydroxy-25D,5α-spirostan-12-one) and sisalagenin (3β-hydroxy-25L,5α-spirostan-12-one), the physical constants of this new sapogenin and its acetate do not coincide with any of those sapogenins and their acetates. From these results, it is considered that this sapogenin is a new compound, and is named laxogenin according to the genus name of the parent plant.

In order to determine the position of the hydroxyl group and the ketone group in laxogenin, the following experiments were carried out.

On Huang-Minlon reduction, laxogenin gave a product, melting at 204~205°, and this was identified as tigogenin by comparison of infrared spectrum and mixed melting point with the authentic specimen. This suggests that the structure of laxogenin is tigogenin having a six-membered ketone group.

*1 Part XLVI. K. Takeda, T. Okanishi, H. Minato, A. Shimaoka : Tetrahedron, in press.
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1) T. Tsukamoto, K. Mitsuhashi : Yakugaku-Kenkyu, **35**, 35 (1963).