[Chem. Pharm. Bull. 13(4) 427~435 (1965)]

UDC 547.891.07 : 547.94.02

55. Shojiro Uyeo, Hiroshi Irie, Akira Yoshitake, and Akira Ito : A Synthesis of Tetrahydrooxocrinine Methine.

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The present work is a part of our project of synthesizing heterocyclic compounds containing a seven-membered nitrogenous ring.

In a previous paper[1] we reported a synthesis of demethyldeoxylycoramine by a sequence of reactions including a crucial Bischler-Napieralski cyclization. Since the yield of the cyclization product containing a seven-membered ring was rather low, we searched for an alternative pathway to the seven-membered ring. Our success using the Schmidt reaction is reported in a separate paper.[2] We have confirmed that the Schmidt reaction with the 1-tetralone (I) gave the 4,5-dihydro-1*H*-2-benzazepin-2(3*H*)-one (Ⅱ), while the 6-methoxy-1-tetralone (Ⅲ) afforded, along with the normal rearrangement product, the methoxy-2-benzazepinone (Ⅳ), and an abnormal product, the methoxy-3-benzazepinone (Ⅴ) in about equal yields. On the basis of these experiments, it became clear that the methoxyl group located in the *p*-position to a carbonyl group played an important role in determining the direction of the rearrangement. We have applied these discoveries to the synthesis of tetrahydrooxocrinine methine (Ⅵ) which represents another example of a compound containing a seven-membered heterocyclic ring. The structure of the methine (Ⅵ) has been elucidated by Wildman[3] on the basis of the synthesis of crinane, the skeleton of crinine (Ⅶ), an Amaryllidaceae alkaloid, and the fact that the tetrahydrooxocrinine methine was optically inactive and hence had a plane of symmetry. A synthesis of this methine would establish unequivocally the

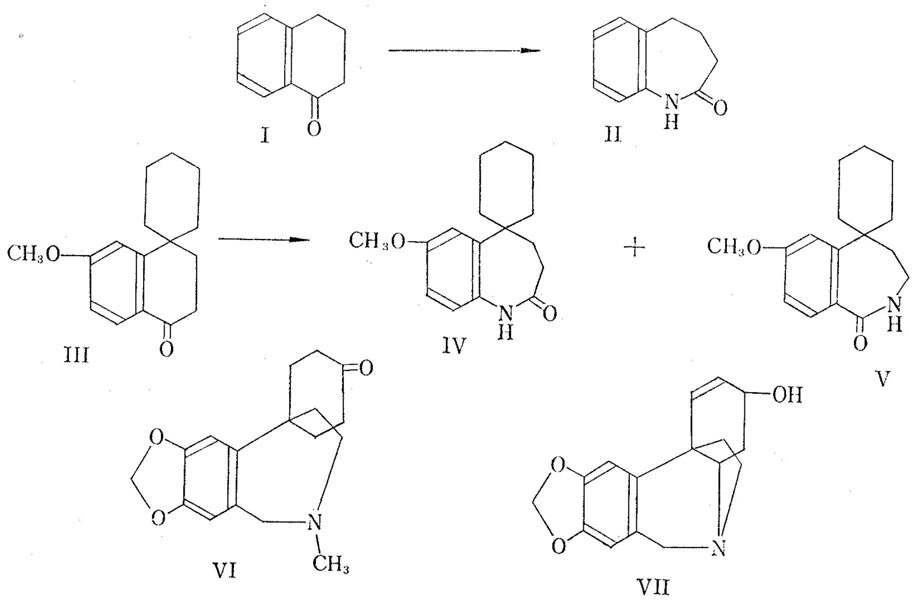

Chart 1.

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1) S. Minami, S. Uyeo : This Bulletin, **12**, 1012 (1964).

2) S. Minami, M. Tomita, H. Takamatsu, S. Uyeo : To be published in this Bulletin.

3) W. C. Wildman : J. Am. Chem. Soc., **80**, 2567 (1958).

location of the hydroxyl group, and thus the structure of crinine itself. The cyanoketone (VIII)[4] served as starting material for the synthesis. The ketone function was protected as the ketal (IX). Alkaline hydrolysis of IX gave an acid (X) which could be reduced directly or through its ester (XI) to the alcohol (XII). Chromic acid converted XII to the aldehyde (XIII) in 30% overall yield.

The Wittig reaction (as modified by Wadsworth and Emmons)[5] provided the acrylate (XIV) in excellent yield. Cope, Knoevenagel, and Reformatsky reactions did not give satisfactory results.

Hydrogenation of XIV in the presence of Adams catalyst, followed by hydrolysis of the ester group, afforded the ketal-carboxylic acid (XVI). Cyclization of the acid chloride derived from the carboxylic acid by the Friedel-Crafts reaction using stannic chloride as catalyst gave a product which showed two carbonyl bands at 1705 and 1660 cm^{-1} indicating that the ketal group in XVI was hydrolysed to the diketone (XVII).

The Schmidt reaction[6] would be ambiguous if carried out with XVII. To circumvent this difficulty, the compound (XVIII) which was obtained by the acid hydrolysis of XVI, was reduced by sodium borohydride to XIX and XX. The observation that XIX was the predominant product may be due to the bulkiness of the three carbon chain which prefers the equatorial orientation. Both the *cis*- and *trans*-isomers gave the same keto-carboxylic acid (XVIII) on oxidation with chromic acid-pyridine. Proof for the stereochemistry of XIX and XX is cited later in this paper.

The *trans*-isomer was acetylated to the acetate (XXI), the acid chloride of XXI was treated with stannic chloride to furnish the acetoxy-1-tetralone (XXII). When subjected to the Schmidt reaction, XXII afforded two isomeric lactams (XXIII) and (XXIV) in almost equal amounts. The structures of XXIII and XXIV were established by reduction of each with lithium aluminum hydride to the corresponding saturated amine. The ultraviolet spectrum of the amine derived from XXIV remained unchanged in acid, while that from XXIII showed a strong hypsochromic shift when acid was added to the neutral ethanolic solution.

Towards the end of our many experiments to obtain a sufficient amount of this compound, we noticed that mild lithium aluminum hydride reduction of the hydroxynitrile (XXVI) or the keto nitrile (VIII) gave an aldimine which was readily hydrolysed with acid to the hydroxy-aldehyde (XXV). The *cis*-configuration of the hydroxyl group and the aldehyde group in XXV was proved by the observation that the corresponding hydroxy-carboxylic acid formed a lactone. A Wittig reaction on this compound, using diethyl ethoxycarbonylmethylphosphonate anion gave predominantly an ester (XXXII), along with some of the desired acrylate (XXVII). Hydrolysis of the former gave a crystalline carboxylic acid, $C_{16}H_{18}O_5$, which contained no double bond conjugated with the carboxyl group, and presumably is represented by formula (XXXIII). The acrylate (XXVII), was hydrogenated over Adams catalyst in ethanol and the resulting propionate (XXVIII) was hydrolyzed in ethanolic sodium hydroxide to give the hydroxy-propionic acid (XXIX) which on oxidation with chromic acid-pyridine complex furnished the keto-carboxylic acid (XVIII) identical with the sample obtained above.

To prevent the formation of XXXII, the hydroxyl group in XXV was protected by acetylation and the resulting acetate (XXX) was then subjected to the same series of reactions as those cited for XXV. The product (XXIX) was acetylated and cyclised to the acetoxytetralone (XXXVI). The Schmidt reaction with this compound gave two seven-membered ring lactams which were not identical with XXIII and XXIV due to the difference in configuration of the hydroxyl group. The desired homoisocarbostyril

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[4] H. Irie, Y. Tsuda, S. Uyeo: J. Chem. Soc., **1959**, 1446.

[5] W. S. Wadsworth, W. D. Emmons: J. Am. Chem. Soc., 83, 1773 (1961).

[6] K. F. Schmidt: Ber., **57**, 704 (1924).

(XXXVIII) could be selected by spectral comparisons with XXIII and XXIV. Hydrolysis of XXXVIII and oxidation provided the ketolactam (XL). The ketone was protected as the ethylene ketal. N-Methylation of XLI gave XLII, which was reduced with lithium aluminum hydride. Hydrolysis of the ketal occurred during the isolation procedure and the final product was XLIII, identical in all respects with an authentic sample of tetrahydro-oxocrinine methine.[*2] This synthesis added further support to the structure of crinine, and could conceivably be extended to the total synthesis of the alkaloid.

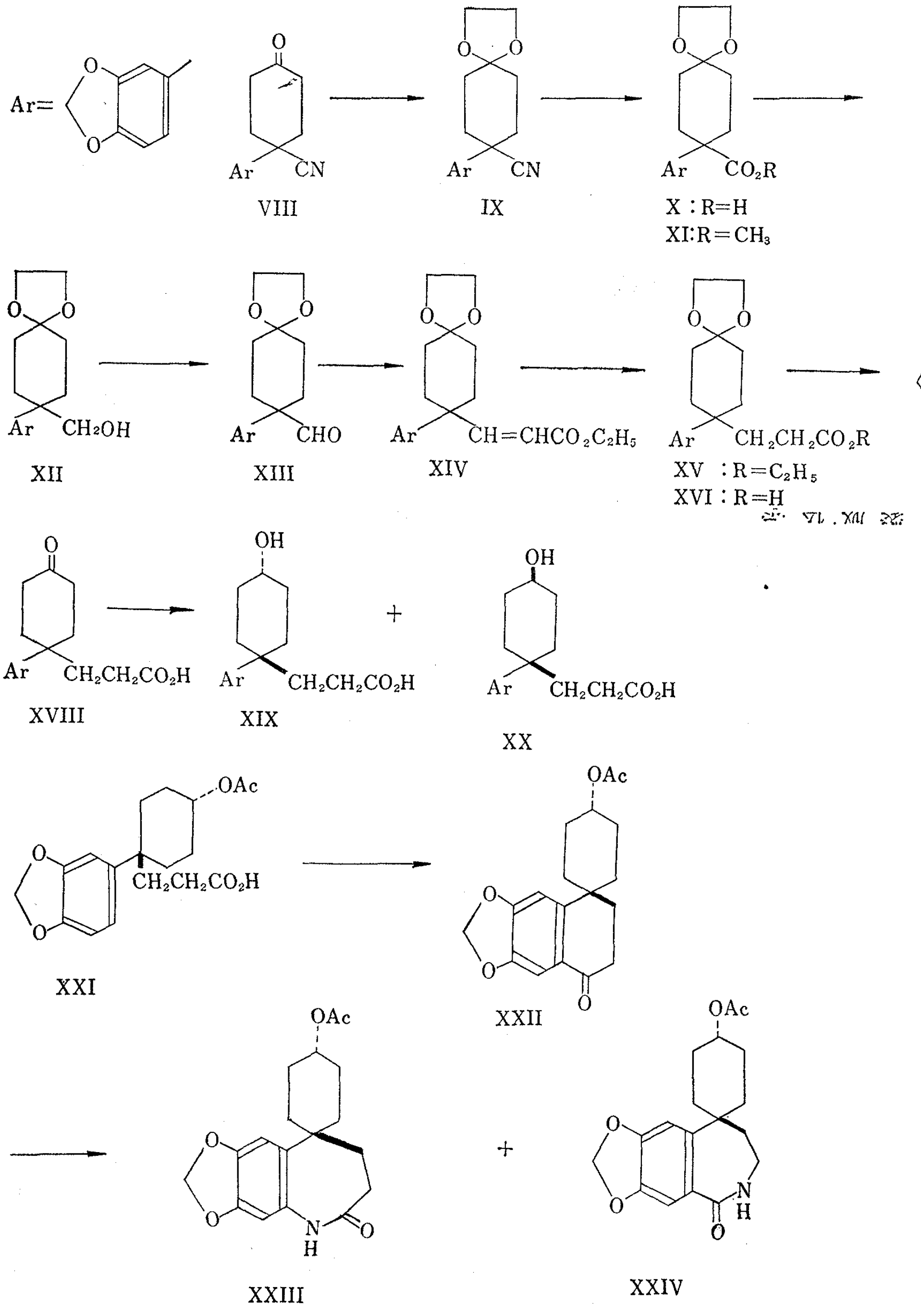

Chart 2.

[*2] We are grateful to Professor W. C. Wildman of the Iowa State University for this sample and an IR spectrum of this compound.

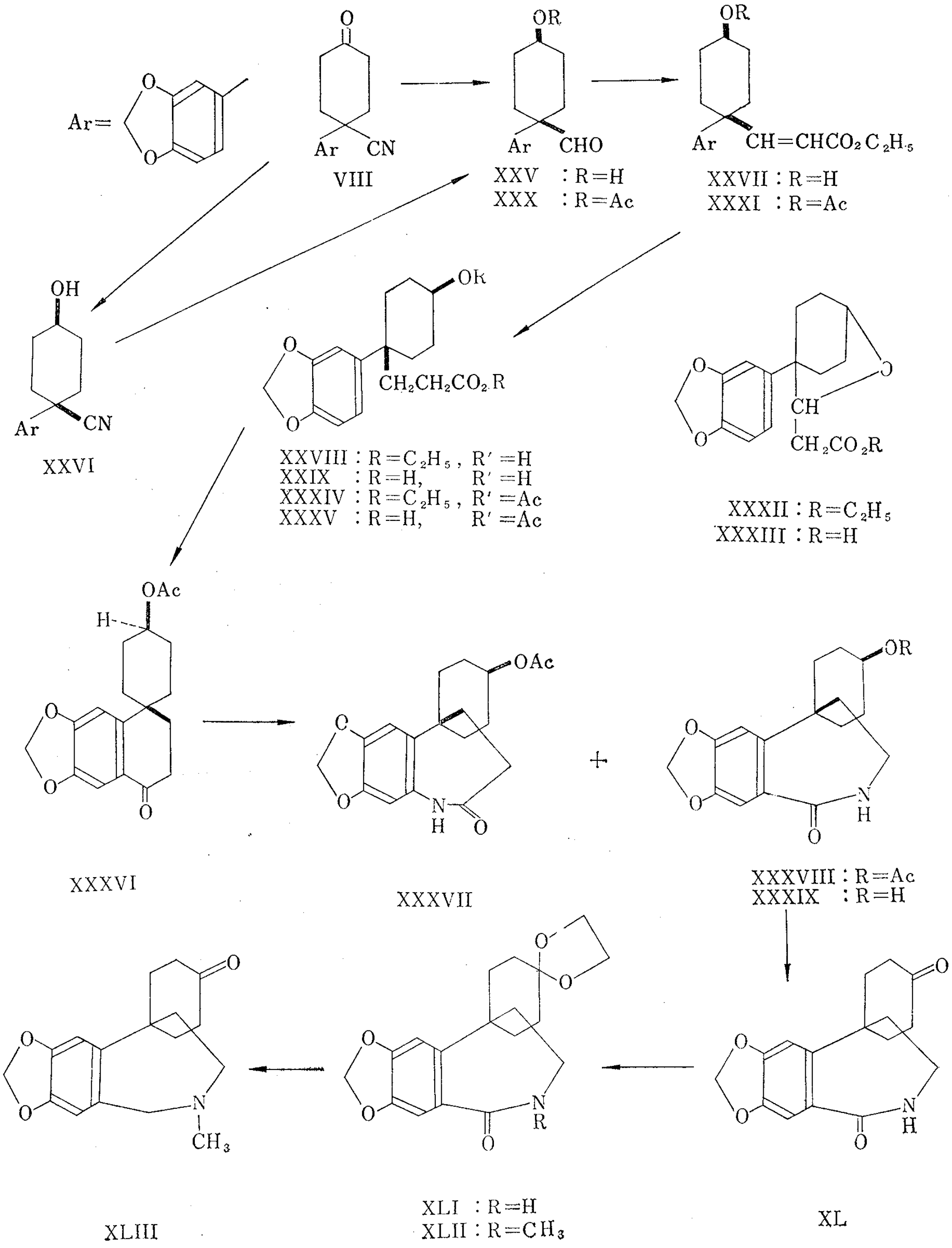

Chart 3.

Experimental

1-(3,4-Methylenedioxyphenyl)-4-oxocyclohexanecarbonitrile Cyclic Ethylene Acetal (IX)——A mixture of the cayno-ketone[4] (Ⅷ) (10 g.), ethylene glycol (50 ml.), boron trifluoride-etherate (5 ml.) and tetrahydrofuran (100 ml.) was kept at room temperature overnight. The solution was poured into aqueous Na_2CO_3 and extracted with C_6H_6. The extract was washed with H_2O, dried and evaporated to dryness to give the cyano-ketone ethylene ketal (10 g.) which crystallized from EtOH as preisms, m.p. 108~109°. IR ν_{max}^{Nujol} cm^{-1}: 2240 (CN). *Anal.* Calcd. for $C_{16}H_{17}O_4N$: C, 66.88; H, 5.96; N, 4.88. Found: C, 66.65; H, 6.12; N, 4.80.

Hydrolysis of the Cyano-ketone Ethylene Ketal (IX)——The cyano-ketone ethylene ketal (Ⅸ) (5 g.) was heated under reflux in diethylene glycol (50 ml.) and 50% aqueous KOH (50 ml.) for 5 hr. The solution was diluted with H_2O (300 ml.) and acidified with concentrated HCl with cooling. The acidic aqueous solution was extracted with ether and the ethereal extract was washed with H_2O, dried and evaporated to dryness to give the ketal-carboxylic acid (X) (4 g.) which crystallized from benzene as needles, m.p. 148~150°. IR$\nu_{\max}^{\text{Nujol}}$ cm^{-1}: 3240~2540 (COOH); 1686 (C=O). *Anal.* Calcd. for $C_{16}H_{18}O_6$: C, 62.74; H, 5.92. Found: C, 62.58; H, 5.74.

Esterification of the Ketal-carboxylic Acid(X)——An excess of ethereal diazomethane was added to a solution of the ketal-carboxylic acid (X) (5 g.) in MeOH (50 ml.) and ether (100 ml.) and the solution was kept at room temperature overnight. After the excess diazomethane had been destroyed by the addition of acetic acid, the solution was washed with aqueous Na_2CO_3 and H_2O, dried and evaporated to dryness to give the ester (XI) (4.5 g) which crystallized from ether-hexane as needles, m.p. 61~62°, IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1}: 1720 (C=O). *Anal.* Calcd. for $C_{17}H_{20}O_6$: C, 63.74; H, 6.29. Found: C, 63.95; H, 6.31.

4-Hydroxymethyl-4-(3,4-methylenedioxyphenyl)cyclohexanone Cyclic Ethylene Actal (XII)——i) A mixture of the ketal-carboxylic acid (X) (3 g.), $LiAlH_4$ (1 g.), and tetrahydrofuran (100 ml.) was heated under reflux for 17 hr. H_2O was added dropwise and the precipitate which formed was removed by filtration. The filtrate was evaporated to dryness to give a residue (3 g.) which was taken up in ether and the ethereal extract was washed with aqueous Na_2CO_3 and H_2O, dried and evaporated to give the ketal-alcohol (XII) (2.7 g.) which crystallized from ether-EtOH as prisms, m.p. 91~92°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 3570 (OH). *Anal.* Calcd. for $C_{16}H_{18}O_5$: C, 65.74; H, 6.90. Found: C, 65.47; H, 7.08.
ii) A solution of the methyl carboxylate (XI) (1 g.) and $LiAlH_4$ (0.1 g.) in tetrahydrofuran (50 ml.) was refluxed for 5 hr. After working up in the usual way, the resulting product was chromatographed in $CHCl_3$ on silica gel. The $CHCl_3$ eluate gave the ketal-alcohol (XII) (0.8 g.) which crystallized from ether-EtOH and had m.p. and mixed m.p. 91~92°. *p*-Nitrobenzoyl derivative of the ketal-alcohol (XII) formed needles (from ether-acetone), m.p. 129~130°. IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1}: 1720 (C=O). *Anal.* Calcd. for $C_{24}H_{19}O_8N$: C, 62.58; H, 5.25; N, 3.17. Found: C, 62.50; H, 5.45; N, 3.16.

1-(3,4-Methylenedioxyphenyl)-4-oxocyclohexanecarboxaldehyde 4-(Cyclic Ethylene Acetal) (XIII)——The foregoing ketal-alcohol (XII) (500 mg.) was added with stirring to the complex prepared from chromium trioxide (500 mg.) and pyridine (5 ml.) and the mixture was kept at room temperature for 24 hr. The mixture was then poured into H_2O and extracted with ether. The ethereal extract was washed with diluted HCl, aqueous Na_2CO_3 and H_2O, dried and evaporated to dryness to give an oil (350 mg.) which was chromatographed in $CHCl_3$ over silica gel. The $CHCl_3$ eluate gave the ketal-aldehyde (XIII) (190 mg.), which was recrystallized from ether-EtOH as prisms, m.p. 68~69°. IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1}: 1720 (C=O). *Anal.* Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 65.99; H, 6.45. The second eluate with chloroform gave the ketal-alcohol (XII) (50 mg.), m.p. and mixed m.p. 91~92°.

Ethyl 1-(3,4-Methylenedioxyphenyl)-4-oxocyclohexaneacrylate Cyclic Ethylene Acetal (XIV)——Triethyl phosphonoacetate (350 mg.) was added dropwise at 15~20° with stirring to a slurry of 50% mineral oil coated NaH (85 mg.) in benzene (5 ml.) and stirring was continued at room temperature until hydrogen evolution had ceased. To this solution, maintained below 25°, was added dropwise the ketal-aldehyde (XIII) (500 mg.) in dry benzene (5 ml.) over a period of 15 min., during which time a gummy solid precipitated. The solution was stirred at room temperature for 45 min. and refluxed for another 30 min., after cooling, a large excess of H_2O was added and the product extracted with ether. The extract, after being washed with H_2O and dried over Na_2SO_4, was evaporated to dryness to give the unsaturated carboxylic ester (XIV) (580 mg.), as prisms, m.p. 101~102° (from EtOH). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1705 (C=O); 1630 (C=C). *Anal.* Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.93; H, 6.70.

Ethyl 1-(3,4-Methylenedioxyphenyl)-4-oxocyclohexanepropionate Cyclic Ethylene Acetal (XV)——The foregoing unsaturated carboxylic ester (XIV) (200 mg.) was hydrogenated over PtO_2 (50 mg.) in EtOH (30 ml.) at room temperature until the calculated amount of H_2 had been absorbed. The mixture was filtered and the filtrate was concentrated to dryness under reduced pressure to give the saturated ester (XV) (155 mg.) which was crystallized from MeOH as prisms, m.p. 75~76°. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1720 (C=O). *Anal.* Calcd. for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23. Found: C, 66.12; H, 7.33.

1-(3,4-Methylenedioxyphenyl)-4-oxocyclohexanepropionic Acid Cyclic Ethylene Acetal (XVI)——The saturated ester (XV) (1 g.) was heated under reflux in 5%-ethanolic NaOH for 2.5 hr. Evaporation of EtOH *in vacuo* gave a solid which was taken up in H_2O (50 ml.). The aqueous solution was acidified with 20% HCl and extracted with ether. The ethereal extract was washed with H_2O, dried and evaporated to give the ketal-acid (XVI) (720 mg.) which crystallized from MeOH-EtOH as prisms, m.p. 131~132°. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 2400~3600 (COOH); 1708 (C=O). *Anal.* Calcd. for $C_{18}H_{22}O_6$: C, 64.65; H, 6.63. Found: C, 64.92; H, 6.68.

Friedel-Crafts Cyclization of the Ketal-carboxylic Acid (XVI)——A solution of the ketal-carboxylic acid (XVI) (300 mg.) and freshly powdered phosphorus pentachloride (200 mg.) in dry benzene (5 ml.) was stirred at 10~15° for 45 min. Stannic chloride (500 mg.) was added to the cooled solution in one portion and stirring was continued at room temperature for a further 10 min. Then ice pieces, ether (5 ml.) and concentrated HCl (0.7 ml.) were added successiviely and the mixture was extracted with ether which was washed with aqueous Na_2CO_3 and H_2O, dried and evaporated to give the diketone (XVII) (90 mg.). Crystallization from ether-EtOH gave prisms, m.p. 188~190° IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1705 (C=O), 1660 (conjugated C=O). *Anal.* Calcd. for $C_{16}H_{16}O_4 \cdot \frac{1}{3}H_2O$: C, 69.06; H, 6.04. Found: C, 69.49; H, 6.06.

1-(3,4-Methylenedioxyphenyl)-4-oxocyclohexanepropionic Acid (XVIII)——The ketal-carboxylic acid (XVI) (300 mg.) in 60% aqueous AcOH (25 ml.) and a few drops of H_2SO_4 was heated on a water-bath for 4 hr. The solution was concentrated under reduced pressure to 8 ml., diluted with H_2O and extracted with ether. The extract was washed with aqueous Na_2CO_3 which was acidified with concentrated HCl and extracted with ether. The ethereal extract, after being washed with H_2O and dried, was evaporated to give the keto-carboxylic acid (XVIII) as colorless prisms (230 mg.), m.p. 138~139° (from ether-EtOH). IR ν_{max}^{KBr} cm^{-1}: 2400~3580 (COOH); 1710 (C=O, COOH). *Anal.* Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 66.08; H, 6.30.

1-(3,4-Methylenedioxyphenyl)-4-hydroxycyclohexanepropionic Acid (XIX) and (XX)——Sodium borohydride (100 mg.) in H_2O (2 ml.) was added to the keto-carboxylic acid (XVIII) (300 mg.) in tetrahydrofuran (25 ml.) and the mixture was stirred at room temparature for 4 hr. The solvent was removed by distillation and the residue taken up in H_2O (20 ml.) which was acidified with dilute HCl and extracted with ether. The extract was washed with H_2O, dried, and evaporated to dryness to afford the *trans*-hydroxy-carboxylic acid (XIX) (200 mg.) which crystallized from ether-EtOH as prisms, m.p. 163~164°. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH); 2450~3200 (COOH); 1708 (C=O). *Anal.* Calcd. for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.71; H, 6.90. The mother liquors were evaporated to dryness to give a residue (50 mg.) which was chromatographed in $CHCl_3$ over SiO_2 gel. The $CHCl_3$ eluate gave the *cis*-hydroxy-carboxylic acid (XX) (40 mg.), m.p. 134~136°, identical in all respects with the sample as described below.

Acetylation of the Hydroxy-carboxylic Acid (XIX)——A mixture of the hydroxy-carboxylic acid (XIX) (150 mg.), pyridine (5 ml.) and Ac_2O (0.5 ml.) was kept at room temperature overnight, then diluted with H_2O, acidified with HCl and extracted with AcOEt. The organric layer was washed with H_2O, dried and evaporated to give the acetoxy-carboxylic acid (XXI) (130 mg.) which crystallized from ether-EtOH as prisms, m.p. 159~160°. IR ν_{max}^{KBr} cm^{-1}: 2400~3600 (COOH); 1720 (acetyl C=O); 1690 (COOH). *Anal.* Calcd. for $C_{18}H_{22}O_6$: C, 64.65; H, 6.63. Found: C, 64.62; H, 6.85.

Friedel-Crafts Cyclization of the Acetoxy-carboxylic Acid (XXI)——Finely powdered phosphorus pentachloride (200 mg.) was added with cooling to a solution of the acetoxy-carboxylic acid (XXI) (200 mg.) in benzene (10 ml.) and the mixture was stirred at 10~15° for 45 min. Stannic chloride (500 mg.) was then added in one portion and the mixture was stirred at room temperature for a further 20 min. Working up in the usual way gave the acetoxy-1-tetralone (XXII) (90 mg.) which was crystallized from ether as prisms, m.p. 142~143°. IR ν_{max}^{Nujol} cm^{-1}: 1720 (C=O); 1670 (conjugated C=O). *Anal.* Calcd. for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.07; H, 6.58.

Schmidt Reaction on the Acetoxy-1-tetralone (XXII)——Sodium azide (40 mg.) was added to a mixture of the acetoxy-1-tetralone (XXII) (100 mg.) and trichloroacetic acid (1.5 g.) at 60~62° and the mixture was stirred at the same temperature for 3 hr. Ice-water was added to the mixture which was made alkaline with aqueous NH_3 and extracted with $CHCl_3$. The $CHCl_3$ extract was dried and evaporated to dryness to give an oil (90 mg.) which was chromatographed in $CHCl_3$ over SiO_2 gel. The first $CHCl_3$ eluate recovered the starting acetoxy-1-tetralone (XXII) (20 mg.), m.p. and mixed m.p. 142~143° unchanged, and the second $CHCl_3$ eluate gave the 2-benzazepinone (XXIII) (25 mg.), m.p. 212~213° which crystallized from ether-EtOH as prisms. IR ν_{max}^{Nujol} cm^{-1}: 3220, 3120 (NH); 1730 (C=O); 1670 (amide). *Anal.* Calcd. for $C_{18}H_{21}O_5N \cdot 1/2H_2O$: C, 63.52; H, 6.52; N, 4.12. Found: C, 63.63; H, 6.39; N, 4.24. UV λ_{max}^{EtOH} mμ: 258, 300. The lactam (3 mg.) was heated in tetrahydrofuran (5 ml.) under reflux with $LiAlH_4$ (10 mg.) for 4 hr. The excess reagent was decomposed by adding ether saturated with H_2O and the solution precipitate which formed was removed by filtration. The filtrate was evaporated to dryness to give a residue which exhibited in the UV spectrum bands at 253, 303 mμ in EtOH and at 243, 290 mμ in ethanolic HCl. The third fraction eluted with the same eluent gave the 3-benzazepinone (XXIV) (25 mg.) as prisms (from EtOH-ether), m.p. 198~199°. IR ν_{max}^{Nujol} cm^{-1}: 3070, 3220 (NH); 1730 (C=O); 1645 (amide). *Anal.* Calcd. for $C_{18}H_{21}O_5N$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.09; H, 6.51; N, 4.20. UV λ_{max}^{EtOH} mμ: 263, 296. This lactam (3 mg.) was reduced with $LiAlH_4$ in the same way as described above. The UV spectra of the resulting amine exhibited absorptions at 235, 290 mμ both in EtOH and in acidic EtOH containing HCl.

Treatment of the *cis*-Hydroxy-nitrile (XXVI) with Lithium Aluminum Hydride——The *cis*-hydroxy-nitrile[4] (XXVI) (300 mg.) and $LiAlH_4$ (50 mg.) in tetrahydrofuran (30 ml.) was refluxed for 4 hr. After cooling, H_2O was added dropwise to the mixture to decompose the excess reagent, the resulting precipitate removed by filtration and the filtrate concentrated to dryness to give a residue which was taken up in $CHCl_3$ and extracted with dilute HCl. The $CHCl_3$ layer gave, on washing with H_2O, drying and evaporation, the unreacted starting material, the *cis*-hydroxy-nitrile (110 mg.). The dilute HCl layer was basified with aqueous NH_3, and extracted with $CHCl_3$. The extract was washed with H_2O, dried and concentrated to give an oil (150 mg.) which was dissolved in AcOH (10 ml.), H_2O (5 ml.) and containing 3 drops of concentrated HCl and heated on a water bath for 1 hr. After cooling, the mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed several times with aqueous Na_2CO_3 and with H_2O, dried, and evaporated to give the *cis*-hydroxy-aldehyde (XXV) (90 mg.), m.p. 124~125° (from EtOH-ether).

Treatment of the Keto-nitrile (VIII) with Lithium Aluminum Hydride——The keto-nitrile (VIII) (500 mg.) in tetrahydrofuran (30 ml.) was refluxed with $LiAlH_4$ (100 mg.) for 5 hr. The excess reagent was decomposed with H_2O and the resulting precipitate was filtered off. The filtrate was evaporated to dryness to give a residue which was taken up in $CHCl_3$. The $CHCl_3$ solution was washed with diluted ice-cold HCl, dried and evaporated to dryness to give a solid which on chromatography in $CHCl_3$ over SiO_2 gel and elution with $CHCl_3$ gave the unchanged keto-nitrile (VIII), m.p. and mixed m.p. 134~135°. Further elution with the same solvent gave the hydroxy-aldehyde (XXV) (50 mg.) which was crystallized from ether-EtOH as prisms, m.p. and mixed m.p. 124~125°. *Anal.* Cacd. for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found : C, 68.03; H, 6.46. IR ν_{max}^{Nujol} cm^{-1} : 3570, 3455 (OH); 1705 (C=O). The aqueous layer separated from the chloroform solution was basified with aqueous NH_3 and extracted with ether. The ethereal extract was washed with H_2O, dried, and evaporated to dryness to give an oil which was taken up in AcOH (10 ml.). The solution was diluted with H_2O (5 ml.) and, after the addition of concentrated HCl (0.3 ml.), heated on a water bath for 30 min. The mixture was diluted with H_2O, extracted with ether and the ethereal extract was washed with aqmeous Na_2CO_3 and H_2O, dried and concentrated to dryness to give the crop of the hydroxy-aldehyde (XXV) (240 mg.), m.p. and mixed m.p. 124~125°.

Wittig Reaction on the *cis*-Hydroxy-aldehyde (XXV)——Triethyl phosphonoacetate (2.3 g.) was added with stirring to a slurry of NaH (540 mg.) (50% in mineral oil) in benzene (50 ml.) at 15~20°. The mixture was stirred at room temperature for 30 minutes and the *cis*-hydroxy-aldehyde (XXV) (2.7 g.) in dry benzene (90 ml.) was then added dropwise to the mixture at 20~25°. Stirring was continued at room temperture for a further 1.5 hr., during which time a brown gummy precipitate appeared. The mixture was then heated under reflux for 30 min., poured into water, and extracted with ether. The ethereal extract was washed with H_2O, dried and evaporated to give an oil (2.5 g.) which was chromatographed in $CHCl_3$ on silica gel. The first $CHCl_3$ eluate gave the compound (XXXII) as an oil (1.5 g.) which exhibited an IR band at 1720 cm^{-1} (unconjugated ester) but no hydroxyl absorption. This oil in EtOH (50 ml.) was hydrolyzed by refluxing with 5% aqueous KOH (20 ml.) for 2 hr. The mixture was concentrated, diluted with H_2O which was washed with ether. After acidification with HCl the mixture was extracted with AcOEt. The extract was washed with H_2O, dried and evaporated to give XXXIII (850 mg.) as prisms, m.p. 178~179° (from ether-EtOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1725 (C=C), 3300~2600 (COOH). *Anal.* Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found : C, 66.45; H, 6.23.

Further elution of the column with $CHCl_3$ afforded the ester (XXVII) as an oil (500 mg.) which showed IR absorptions at 3450 (OH), 1695 (unsaturated ester) and 1635 cm^{-1}(C=C) in $CHCl_3$.

Hydrogenation of the *cis*-Hydroxy-acrylate (XXVII)——The *cis*-hydroxy-acrylate (XXVII) (300 mg.) in EtOH (50 ml.) was hydrogenated over PtO_2 (50 mg.). The catalyst was filterd off and the filtrate was evaporated under a vacuum to give an oil which was chromatographed in $CHCl_3$ over silica gel. The $CHCl_3$ eluate gave the oily hydroxy propionate (XXVIII) (280 mg.). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3450 (OH); 1720 (C=O).

Hydrolysis of the Hydroxy-propionate (XXVIII)——The hydroxy-propionate (XXVIII) (300 mg.) was refluxed in 5% ethanolic NaOH (50 ml.) for 2.5 hr. After removal of EtOH by evaporation under reduced pressure, the residue was taken up in H_2O, and the aqueous solution was shaken with ether. The aqueous layer was then acidified with dilute HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried and evaporated to dryness to give the hydroxy-propionic acid (XXIX) (270 mg.) which crystallized from ether-EtOH as prisms, m.p. 134~136°. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH); 2600~3200 (COOH); 1720 (C=O). *Anal.* Calcd. for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found : C, 65.52; H, 6,85.

Oxidation of the Hydroxy-propionic Acid (XXIX)——The hydroxy-acid (XXIX) (100 mg.) in CrO_3 (100 mg.)-pyridine (1 ml.) complex was kept at room temperature overnight. The mixture was diluted with H_2O, made acidic with concentrated HCl, and extracted with ether. The ethereal extract was dried and concentrated to dryness to give the keto-carboxylic acid (XVIII) (70 mg.), m.p. and mixed m.p. 138~139° (from ether-EtOH).

Acetylation of the *cis*-Hydroxy-aldehyde (XXV)——The *cis*-hydroxyaldehyde (XXV) (100 mg.) in pyridine (3 ml.) and Ac_2O (1 ml.) was kept at room temperature overnight. The mixture was diluted with ice-H_2O and extracted with ether. Working up in the usual way afforded the *cis*-acetoxy-aldehyde (XXX) as prisms (85 mg.), m.p. 104~106° (from ether-EtOH). IR ν_{max}^{KBr} cm^{-1} : 1715 (AcO, C=O). *Anal.* Calcd. for $C_{16}H_{20}O_5$: C, 66.19; H, 6.25. Found : C, 65.96; H, 6.29.

Wittig Reaction on the *cis*-Acetoxy-aldehyde (XXX)——Triethyl phosphonoacetate (250 mg.) was added to the suspension of NaH (60 mg.) in benzene (5 ml.) at 20°. The mixture was stirred at 20~25° for 30 min. and then a solution of the *cis*-acetoxy-aldehyde (XXX) (200 mg.) in benzene (10 ml.) was added dropwise with stirring to the mixture at 20~25°. Stirring was continued at room temperature for 1.5 hr. and under reflux for a further 30 min. Ether was added to the mixture and the solution was washed with H_2O, dried and evaporated to give *cis*-acetoxy-acrylate (XXXI) (180 mg.) which was crystallized from EtOH as prisms, m.p. 92~93°. IR ν_{max}^{Nujol} cm^{-1} : 1705 (C=O); 1635 (C=C). *Anal.* Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found : C, 66.36; H, 6.89.

Hydrogenation of the *cis*-Acetoxy-acrylate (XXXI)——Hydrogenation of the *cis*-acetoxy-acrylate (XXXI) (180 mg.) in EtOH (40 ml.) was carried out in the presence of PtO_2 (50 mg.) as catalyst until hydrogen uptake (12 ml.) had ceased. The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to give an oil (150 mg.) which was chromatographed in $CHCl_3$ on silica gel. The $CHCl_3$ eluate gave an oil (XXXIV) which could not be induced to crystallize. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (C=O).

Hydrolysis of the *cis*-Acetoxy-propionate (XXXIV)——The *cis*-acetoxy-propionate (XXXIV) (150 mg.) was heated on a water bath in EtOH (25 ml.) and 25% aqueous NaOH (3 ml.) for 2 hr. EtOH was removed under reduced pressure, and the residue was diluted with H_2O, washed with ether, acidified with concentrated HCl, and extracted with AcOEt. The organic layer was washed with H_2O, dried and evaporated to dryness to give the *cis*-hydroxy-propionic acid (XXIX) (100 mg.), m.p. and mixed m.p. 134~136°.

***cis*-Acetoxy-propionic Acid (XXXV)**——A mixture of the *cis*-hydroxy-propionic acid (XXIX) (450 mg.), pyridine (10 ml.) and Ac_2O (1.5 ml.) was kept at room temperature overnight. After the addition of ice-H_2O, the mixture was acidified with concentrated HCl and extracted with AcOEt. The extract was washed with dilute HCl, and H_2O, dried, and evaporated to give the acetate (XXXV) (420 mg.). After purification by chromatography in $CHCl_3$ on silica gel, the compound formed prisms, m.p. 127~128° (from ether-EtOH). IR ν_{max}^{KBr} cm^{-1}: 3300~2600 (COOH), 1720 (C=O), and 1715 (C=O). *Anal.* Calcd. for $C_{18}H_{22}O_6$: C, 64.65; H, 6.63. Found: C, 64.46; H, 6.73.

Friedel-Crafts Cyclization of the Acetoxy-propionic Acid (XXXV)——Finely powdered phosphorous pentachloride (2 g.) was added with cooling with ice-H_2O to the acetoxy-propionic acid (2 g.) in benzene (100 ml.) and the mixture was stirred for 1 hr. Stannic chloride (5 g.) was added to the mixture in one portion and the stirring was continued at 0° for 45 min. and then at room temperature for an additional 20 min. The mixture was poured into ice containing concentrated HCl, and extracted with ether. The organic layer was washed with aqueous Na_2CO_3 and H_2O, dried, and concentrated to give the acetoxy-1-tetralone (XXXVI) (1.3 g.) which was crystallized from benzene as prisms, m.p. 219~222°. IR ν_{max}^{KBr} cm^{-1}: 1720 (AcO); 1670 (conjugated C=O). *Anal.* Calcd. for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.61; H, 6.38.

Schmidt Reaction on the Acetoxy-1-tetralone (XXXVI)——NaN_3 (120 mg.) was added with stirring to a mixture of the acetoxy-1-tetralone (XXXVI) (300 mg.) and CCl_3CO_2H (5 g.) at 60~62°. Stirring was continued at 60° for 4 hr. and the mixture was poured into ice-cold 30% aqueous NH_3, and extracted with $CHCl_3$. The chloroform extract was washed with H_2O, dried and evaporated to dryness to give a residue (290 mg.) which was chromatographed in $CHCl_3$ on silica gel. The first $CHCl_3$ eluate gave the unchanged starting material (XXXVI) (90 mg.), m.p. and mixed m.p. 219~222°. The second eluate with the same solvent gave the 2-benzazepinone (XXXVII) (90 mg.) which was crystallized from EtOH-ether as prisms, m.p. 223~224°. UV λ_{max}^{EtOH} $m\mu$ ($\log \varepsilon$): 256 (3.82); 297 (3.76). IR ν_{max}^{KBr} cm^{-1}: 3220, 3110 (NH); 1720 (acetyl C=O); 1668 (amide C=O). *Anal.* Calcd. for $C_{18}H_{21}O_5N$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.04; H, 6.55; N, 4.00.

A small sample of this compound was reduced by $LiAlH_4$ in boiling tetyahydrofuran. The resulting base exhibited in the ultraviolet spectrum absorption maxima at 253 and 303 $m\mu$ in EtOH and 243 and 290 $m\mu$ in a HCl solution.

The third eluate with the same solvent gave the 3-benzazepinone (XXXVIII) (95 mg.). Crystallization from EtOH-ether gave colourless prisms, m.p. 104~105°. UV λ_{max}^{EtOH} $m\mu$ ($\log \varepsilon$): 262 (3.47); 294 (3.49). IR ν_{max}^{KBr} cm^{-1}: 3210, 3050 (NH); 1720 (acetyl C=O); 1655 (amide C=O). *Anal.* Calcd. for $C_{18}H_{21}O_5N$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.07; H, 6.65; N, 4.22.

A small sample of this compound was reduced as above to give a base which showed in the UV spectrum absorption maxima at 235 (shoulder) and 290 $m\mu$ both in EtOH and HCl solution.

Hydrolysis of the Acetoxy-lactam (XXXVIII)——A solution of the acetoxy-lactam (XXXVIII) (200 mg.) in EtOH (20 ml.) and 5% aqueous K_2CO_3 (4 ml.) was heated under reflux for 20 min., then concentrated under reduced pressure and diluted with H_2O. The aqueous solution was acidified with diluted HCl and extracted with $CHCl_3$, which was washed with H_2O, dried and evaporated to dryness to give the hydroxy-lactam (XXXIX) (180 mg.). Crystallization from EtOH-ether gave prisms, m.p. 249~250°. IR ν_{max}^{KBr} cm^{-1}: 3490, 3260 (OH and NH); 1655 (amide C=O). *Anal.* Calcd. for $C_{16}H_{19}O_4N$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.65; H, 6.73; N, 4.82.

Oxidation of the Hydroxy-lactam (XXXIX)——The hydroxy-lactam (XXXIX) (100 mg.) was added to the CrO_3 (100 mg.)-pyridine (1 ml.) complex and the mixture was kept at room temperature overnight. After dilution with H_2O, the mixture was extracted with $CHCl_3$, the $CHCl_3$ extract washed with successively with dilute HCl, aqueous Na_2CO_3 and H_2O, dried and evaporated to dryness to give the keto-lactam (XL) (80 mg.) which was crystallized from ether-acetone as prisms, m.p. 114~115°. IR ν_{max}^{KBr} cm^{-1}: 3200, 3040 (NH); 1705 (six-membered C=O); 1650 (amide C=O). *Anal.* Calcd. for $C_{16}H_{17}O_4 \cdot \frac{1}{2}H_2O$: C, 64.85; H, 6.12; N, 4.73. Found: C, 64.93; H, 6.59; N, 4.91.

Ketalization of the Keto-lactam (XL)——A solution of the keto-lactam (XL) (50 mg.) and boron trifluoride-etherate (3 drops) in tetrahydrofuran (2 ml.) and ethyleneglycol (1 ml.) was kept at room temperature overnight, then poured into H_2O and extracted with $CHCl_3$. The organic layer was washed with aqueous Na_2CO_3 and H_2O, dried and concentrated to dryness to give the ketal-lactam (XLI) (45 mg.) which was crystallized from EtOH-acetone as prisms, m.p. 259~261°. IR ν_{max}^{KBr} cm^{-1}: 3225, 3065 (NH); 1658 (amide C=O). *Anal.* Calcd. for $C_{18}H_{21}O_5N$: C, 64.36; H, 6.45; N, 4.17. Found: C, 64.24; H, 6.33; N, 4.07.

N-Methylation of the Ketal-lactam (XLI)——A mixture of the ketal-lactam (XLI) (100 mg.) and NaH (80 mg.) (50% in mineral oil) in dry benzene (30 ml.) was heated under reflux for 2 hr. After cooling, methyl iodide (1 ml.) was added to the benzene solution which was refluxed for 1 hr. The reaction mixture was washed with H_2O, dried and evaporated to dryness to give the N-methyl-ketal-lactam (XLII) (85 mg.) which was crystallized from acetone-ether as prisms, m.p. 179~180°. IR ν_{max}^{KBr} cm^{-1}: 1640 (amide C=O). *Anal.* Calcd. for $C_{19}H_{23}O_5N$: C, 66.07; H, 6.71; N, 4.06. Found: C, 65.79; H, 6.80; N, 4.07.

Tetrahydrooxocrinine Methine (XLIII)——A solution of the N-methyl-ketal-lactam (XLII) (70 mg.) and $LiAlH_4$ (80 mg.) in tetrahydrofuran (30 ml.) was heated under reflux for 5 hr. The excess reagent was decomposed by addition of a small amount of H_2O and the precipitate which formed was removed by filtration. The filtrate was concentrated to dryness to give a solid which was taken up in $CHCl_3$. The $CHCl_3$ solution was extracted with diluted HCl and the aqueous layer was basified with aqueous NH_3, and extracted with ether. The ethereal extract was washed with H_2O, dried and evaporated to dryness to afford tetrahydrooxocrinine methine (XLIII) (50 mg.) which crystallized from EtOH-ether as prisms, m.p. 147~148°, identical in all respects with an authentic sample. UV λ_{max}^{EtOH} mμ (log ε): 242 (3.62), 290 (3.58). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705 (C=O). *Anal.* Calcd. for $C_{17}H_{21}O_3N$: C, 71.05; H, 7.37; N, 4.87. Found: C, 71.33; H, 7.45; N, 4.93.

Summary

Tetrahydrooxocrinine methine (XLIII) has been synthesized by a sequence of reactions including ring enlargement of a tetralone (XXXVI) to a seven-membered nitrogenous ring compound (XXXVIII) by the use of Schmidt reaction.

(Received November 20, 1964)

[Chem. Pharm. Bull. 13(4) 435~440 (1965)]

UDC 547.918 : 615.711.5

56. Toshio Kawasaki, Itsuo Nishioka, Tatsuo Yamauchi, Kazumoto Miyahara, and Miyoko Enbutsu: Digitalis Saponins. III.[*1] Enzymatic Hydrolysis of Leaf Saponins of *Digitalis purpurea* L.

(*Faculty of Pharmaceutical Sciences, Kyushu University*[*2])

In the preceding paper[*1] of this series it was reported that the crude saponin of *Digitalis purpurea* leaves precipitated by cholesterol from the butanol extracts of the remainings from the manufacture of cardiac glycosides contained two saponins (I and II). I was composed of tigogenin- and gitogenin-tetraglycosides (desgalactotigonin and F-gitonin) and II was a mixture of three pairs of more polar oligosides each of which seemed also to consist of tigogenin- and gitogenin-glycosides. Since I and its component saponins had[1] the greater capacity than II and commercial "digitonin" in forming molecular compound of great insolubility with cholesterol, it was desired to obtain enough amount of I in order to investigate the structures and properties of its component saponins in detail. This paper concerns a convenient method to prepare I, and subsequently the component saponins, from the crude saponin and more efficiently from the original butanol extract by a specific partial hydrolysis of II with commercially available enzyme preparations.

[*1] Presented at the 84th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 5, 1964. Part II. T. Kawasaki, I. Nishioka: This Bulletin, **12**, 1311 (1964).

[*2] Katakasu, Fukuoka (川崎敏男, 西岡五夫, 山内辰郎, 宮原一元, 円仏美代子).

[1] Presented at the 82nd Annual Meeting of Pharmaceutical Society of Japan, Shizuoka, Nov. 3, 1962.

The content of I in the crude saponin[*1] was less than that of II, the isolation of I by alumina chromatography as reported[*1] is not always practical for a large scale experiment, and I has remained relatively inaccessible. However, co-existence of I and II suggests that the former might be the prosapogenin of the latter and II could be converted enzymatically[*3] into I. As a preliminary experiment, when II obtained by alumina chromatography was treated in aqueous alcohol with emulsin, snail enzyme, hemicellulase and amylase and the products were examined by paper chromatography, II was found to be cleaved to give a saponin (tentatively represented as FSE) corresponding to I and glucose. The experiment was then conducted in a larger scale and a considerable amount of amorphous precipitates[*3] was obtained as a product. The product was extracted with chloroform-methanol (1:1) mixture, the solution was concentrated and left to stand to give a crystalline solid which revealed one spot of FSE on paper chromatograms and was shown on thin-layer of silica gel G to be composed of two saponins (FSE-1 and -2) which correspond very likely to desgalactotigonin and F-gitonin (Fig. 1). The mixture was separated on cellulose powder impregnated with formamide[*1] or preferably on silica gel column[*4] into FSE-1, fine needles, m.p. 284~286° (decomp.), $[\alpha]_D^{28}$ $-68°$ ($c=0.50$, pyridine), and FSE-2, fine needles, m.p. 251~255° (decomp.), $[\alpha]_D^{28}$ $-66°$ ($c=0.50$, pyridine).

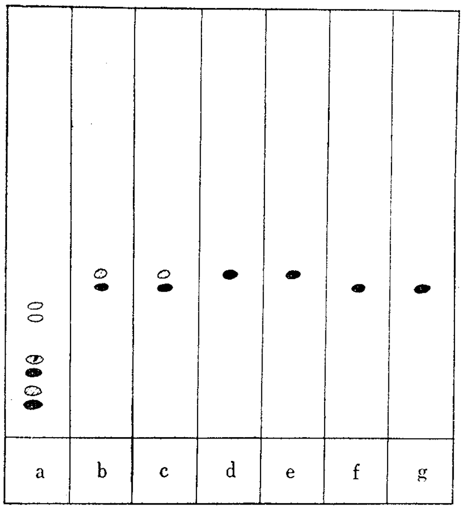

Fig. 1. Thin-layer Chromatorams on Silica Gel G

Solvent : $CHCl_3$-MeOH-H_2O (65:35:10)

a: Saponin II[*1]
b: FSE
c: Saponin I[*1]
d: FSE-1
e: Desgalactotigonin[*1]
f: FSE-2
g: F-Gitonin[*1]

By acid hydrolysis followed by qualitative and quantitative determinations of the products they were found to be tigogenin- and gitogenin-glycosides both of which had the same kind and number of monosaccharide units (2 glucose+galactose+xylose) and were identified with desgalactotigonin and F-gitonin, respectively, by direct comparisons with the authentic samples.[*1] Accordingly it was proved that FSE is nothing but I and that desgalactotigonin and F-gitonin are the prosapogenins of II and selectively yielded by enzymatic cleavage from the corresponding parent saponins.

In the above experiment it was noted that the tetraglycosides formed were not likely to undergo further degradation[*3,*5] and the method seemed applicable to a mix-

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[*3] M. M. Krider, J. R. Branaman, M. E. Wall (J. Am. Chem. Soc., **77**, 1238 (1955)) have reported that the partial hydrolysis of steroid saponin of *Yucca schidigera* (water-soluble) with native enzymes or dil. HCl or H_2SO_4 produced water-insoluble prosapogenins, and that the products of enzymatic cleavage (consisting primarily of sarsasapogenin linked to glucose-galactose oligosides) were obtained in the crystalline form. An attempted partial hydrolysis of II with acid resulted in providing less polar compounds together with I and II. Kikuba-saponin (diosgenin + 3 glucose + rhamnose) of *Dioscorea septemloba* Thunb. was cleaved with emulsin to give gracillin (diosgenin + 2 glucose + rhamnose) as precipitates from the reaction mixture (T. Kawasaki, T. Yamauchi, R. Yamauchi : This Bulletin, **10**, 698 (1962)).

[*4] Chromatography on silica gel was advantageous over that on formamide-impregnated cellulose powder in easiness of the procedure and in resolving power of the component saponins. According to the private communication (March 6, 1964) from Prof. Tschesche, some saponin mixtures were successfully separated by silica gel chromatography also in his laboratory.

[*5] In part, at least, it may be due to the insolubility of the product. The BuOH extract and the crude saponin from the leaves harvested in September contained originally a trace amount of less polar compound (substance (x)) besides I and II (cf. Fig. 2a, b, c).

ture of I and II. Hence the hydrolysis of the crude saponin was carried out in the same way and I was successfully obtained in 27～53% yield.[*6]

Meanwhile, a treatment of the original butanol extract with decolorizing carbon in methanol provided a highly hygroscopic (giving a clear aqueous solution) yellowish-brown powder (Ex) which showed a thin-layer chromatogram (Fig. 2c) quite similar[*5,*7] to that (Fig. 2b) of the crude saponin. Therefor the direct use of the extract was thought to offer a more efficient and economical method for the preparation of I. When an aqueous solution of Ex was allowed to stand with a commercial amylase or cellulase preparation, the reaction mixture yielded precipitates quite analogously to the above experiment for the crude saponin. They were extracted with chloroform-methanol (extract: TLC, Fig. 2d; yield, 24～40%) and recrystallized to give I revealing only two spots of the expected teraglycosides on thin-layer (Fig. 2e).

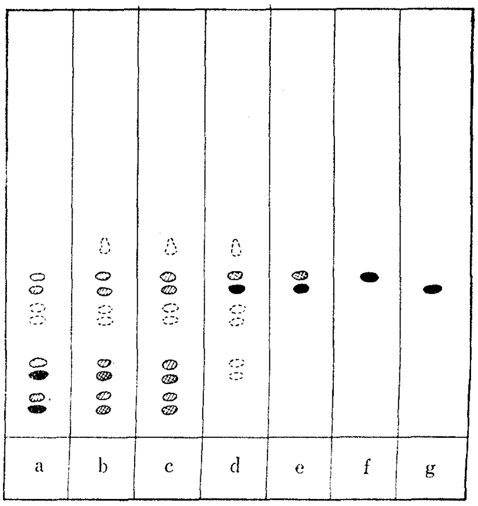

Fig. 2. Thin-layer Chromatograms on Silica Gel G

Solvent: $CHCl_3$-MeOH-H_2O (65:35:10)
a: Crude saponin (Oct.-Nov.)[*1,8]
b: Crude saponin (Sept.)[*5,8]
c: BuOH extract (Sept.)[*5,8] treated with carbon in MeOH (Ex)
d: $CHCl_3$-MeOH extract of a product of enzymatic cleavage of (b) or (c)
e: FSE=Saponin I
f: FSE-1=Desgalactotigonin
g: FSE-2=F-Gitonin

I thus obtained from the crude saponin or from the butanol extract was fractionated into desgalactotigonin and F-gitonin by silica gel chromatography as mentioned afore, and when the latter is contained in I about 85%,[*8] pure F-gitonin was provided only by repeated recrystallization of I from dilute ethanol and from butanol saturated with water.

Thereby saponin (I) in the *Digitalis purpurea* leaves, accordingly also the two component tetraglycosides, particularly F-gitonin, became available in large amounts.

Recently Tschesche and Wulff have reported[2] that the hydrolyses of "digitonin" (Merck) (seed saponins of *Digitalis purpurea*) in water with four kinds of enzyme preparations resulted in a slight spliting (by β-glucosidase and "Luizyme") of xylose, glucose and galactose. The difference in behaviors of seed- and leaf-saponins against enzyme is worth of note.

[*6] In one case when a sample was cleaved in H_2O, I was obtained in up to 60% yield (cf. experimental part).

[*7] Several unknown substances[*1] detected on paper chromatograms of the original BuOH extract were almost removed by the treatment with carbon in MeOH. The relative amounts of the tetraglycosides (*vs.* the polar oligosides) in Ex was apparently less than those in the crude saponin (cf. preceding paper[*1]).

[*8] The content (purity) was calculated from the yields of gitogenin and tigogenin obtained by hydrolysis of a saponin mixture followed by alumina chromatographic separation of the sapogenin fraction. The yield of gitogenin from the BuOH extract or Ex was almost same as those from crude saponin[*1] precipitated by cholesterol and from enzymatically prepared I (=FSE), while it varied to a considerable extent depending on the harvest time of the leaves: leaves harvested in September, gitogenin ca. 60%; those harvested in October～November, ca. 85% (cf. Fig. 2a, b). F. Weiss and O. Manns (Pharm. Zentralhalle, **98**, 437 (1959)) have reported that "tigonin" was the major component of a leaf saponin (yield of sapogenin: tigogenin, 40～70%; gitogenin, 20～30%).

2) R. Tschesche, G. Wulff: Tetrahedron, **19**, 621 (1963).

Experimental[*9]

Enzymatic Hydrolysis of Saponin (II)——As a preliminary experiment, II (50 mg.) was incubated with an enzyme, the reaction mixture was diluted with H_2O, extracted with BuOH, and BuOH and H_2O layers were respectively evaporated *in vacuo* and examined by the conventional paper chromatography (PC). The results are summarized in Table I.

Table I. Enzymatic Hydrolysis of Saponin (II) (50 mg.)

Medium (ml.)	Enzyme (mg.)	Time (days)	Temp. (°C)	Products BuOH layer (saponin)	 H_2O layer (sugar)
30% EtOH 100 AcOH 0.2	emulsin[a] 500	5	25	FSE[e] II (trace)	glucose[f]
30% EtOH 100	snail enzyme[b] 200	5	25	FSE	glucose
20% EtOH 50 AcOH 0.1	hemicellulase[c] 100	7	25	〃	〃
20% EtOH 50 AcOH 0.1	"takadiastase-A"[d] 100	7	30	〃	〃

a) Prepared from the apricot seeds according to the Helferich's procedure (B. Helferich, *et al.*: Z. physiol. Chem., **208**, 91 (1932); **209**, 369 (1932); **215**, 277 (1933).

b) Kindly furnished by Dr. M. Shimizu of Daiichi Seiyaku Co., Ltd., to whom the authors are grateful.

c) Commercical preparation (Tokyo Kasei Co., Ltd.).

d) Kindly furnished by Dr. M. Iwai of Sankyo Co., Ltd., to whom the authors are grateful.

e) Stand for the product of Rf 0.45 on paper chromatograms[*1] (solv., BuOH-AcOH-H_2O (4:1:5); Rf values of reference compounds, saponin (I) 0.45, saponin (II) 0.24).

f) Examined by paper chromatography.[*1]

Subsequently II (gitogenin glycoside content: ca. 85%)[*8] (2 g.) was dissolved in 20% EtOH (500 ml.), and AcOH (0.2 ml.), toluene (0.3 ml.) and hemicellulase or "takadiastase-A" (4 g.) were added. The mixture was allowed to stand at 30° for 7 days. The amorphous precipitates formed were collected by filtration, dried, extracted with $CHCl_3$-MeOH (1:1) and the solution was concentrated and left stand to give a crystalline solid (FSE) (gitogenin glycoside: ca. 85%) (yield, 930 mg.; PC Rf 0.45; TLC, Fig. 1). From another sample of II (gitogenin glycoside: ca. 60%)[*8] FSE (gitogenin glycoside: ca. 60%) was similarly obtained.

Fractionation of the Product (FSE) into the Component Saponins (FSE-1 and -2)——Cellulose powder chromatography: FSE (150 mg.) above obtained (gitogenin glycoside: ca. 60%) was separated into four fractions by chromatography on cellulose powder impregnated with formamide in the same way as reported before[*1] using $CHCl_3$-tetrahydrofuran-pyridine (10:10:2)/formamide (4) as the solvent. Fr. 2 (TLC; FSE-1 +, FSE-2 −) was recrystallized from CH_2Cl_2-MeOH to give a crystalline powder (12 mg.). Further recrystallization from $CHCl_3$ (or CH_2Cl_2)-MeOH afforded thin-layer chromatographically pure FSE-1 as a crystalline powder, m.p. 284~286° (decomp.). Fr. 4 (TLC; FSE-1 −, FSE-2 +) was recrystallized from CH_2Cl_2-MeOH and from BuOH saturated with H_2O (BuOH-H_2O) to give pure (TLC) FSE-2 as fine needles (18 mg.), m.p. 252~255° (decomp.).

Silica gel chromatography: FSE (500 mg.) in $CHCl_3$-MeOH-H_2O (7:3:1) (bottom layer) (3 ml.) was placed on a silica gel (Kantō Kagaku Co., Ltd., 100~200 mesh, 250 g.) column (diameter, 5 cm.), eluted with the same solvent, fractionated into 416 portions and each portion (ca. 5 ml.) was examined by TLC: Fr. 1~240, none; Fr. 241~260, 3 mg., unknown substance (x);[*5] Fr. 261~265, 25 mg., x ±, FSE-1 +; Fr. 266~300, 116 mg., FSE-1; Fr. 301~310, 29 mg., FSE-1 +, FSE-2 ±; Fr. 311~325, 27 mg., FSE-1 +, FSE-2 +; Fr. 326~337, 62 mg., FSE-1 ±, FSE-2 +; Fr. 338~390, 178 mg., FSE-2; Fr. 391~416, 47 mg., FSE-2 +, polar substance ±. Fr. 266~300 was recrystallized from dil. MeOH to give thin-layer chromatographically pure FSE-1 as fine needles, m.p. 284~286° (decomp.), $[\alpha]_D^{28}$ −68° (c=0.5, pyridine). Fr. 338~390 was recrystallized from dil. MeOH or BuOH-H_2O to afford pure (TLC) FSE-2 as fine needless, m.p. 251~255° (decomp.), $[\alpha]_D^{28}$ −66° (c=0.5, pyridine).

[*9] Paper chromatography (PC) of saponins, sopogenins and sugars, thin-layer chromatography (TLC) of saponins, hydrolysis of saponins and qualitative and quantitative determinations of the products were all carried out in the same ways as described before.[*1] All melting points were taken on a Kofler block and are uncorrected.

FSE-1——Acid hydrolysis of FSE-1 (6.6 mg.) yielded tigogenin (2.6 mg.), and xylose, glucose and galactose in a ratio of 1:1.7:1 (calcd. for desgalactotigonin dihydrate: tigogenin, 2.64 mg.; sugar ratio, xylose-glucose-galactose=1:2:1). Indentified with desgalactotigonin[*1] by mixed melting point, comparisons of $[\alpha]_D$ and IR spectra and co-chromatography on thin-layer.

FSE-2——Acid hydrolysis of FSE-2 (10.0 mg.) yielded gitogenin (4.3 mg.), and xylose, gluose and galactose in a ratio of 1:1.8:0.9 (calcd. for F-gitonin dihydrate: gitogenin, 3.98 mg.; sugar ratio, xylose-glucose-galactose=1:2:1). Identical with F-gitonin[*1] in every respect.

Enzymatic Hydrolysis of the Crude Saponin (a Mixture of Saponins (I and II))——Crude saponin (gitogenin glycoside: ca. 85%) (TLC, Fig. 2a) (10 g.) obtained *via* molecular compound with cholesterol was dissolved in 20% EtOH (3 L.) containing AcOH (3 ml.) and toluene (2 ml.) and the solution was treated with "takadiastase-A" or hemicellulase (20 g.) at 30° for 8 days. The reaction mixture (amorphous substances being deposited) was diluted with H_2O (1 L.) and the precipitates were collected by filtration, extracted with $CHCl_3$-MeOH and the solution was concentrated and left stand to give FSE (=I) as a crystalline powder (3.9 g.), PC, Rf 0.45; TLC, Fig. 2e. In several runs the yields were in the range of 27~53%. Another sample of crude saponin (gitogenin glycoside: ca. 60%) (TLC, Fig. 2b) provided FSE in 45% yield in the same way as above, and the same sample in H_2O (1%) with "takadiastase-A" (half amount as much as that of the saponin) gave FSE in about 60% yield.

Enzymatic Hydrolysis of the Butanol Extract of the Remainings from the Manufacture of Cardiac Glycosides——BuOH extract (25 g.) in 90% MeOH (250 ml.) was refluxed with decolorizing carbon (5 g.) for 1 hr., carbon was filtered off and the filtrate was evaporated *in vacuo* to provide an yellowish-brown, highly hygroscopic powder (Ex) (21 g.). It gave a clear aqueous solution and the thin-layer chromatogram as shown in Fig. 2c. An aq. solution of Ex was subjected to enzymatic hydrolyses at 37° in various conditions: concentration of Ex, 0.5~10%; kind and amount of enzyme preparation, "takadiastase-A," "-B,"[*10] hemicellulase, "sanactase"[*11] and "meicelase,"[*12] twice~one-twentieth weight as mucn as that of Ex; incubation time, 1~4 days. The precipitates separated out from the reaction mixture were collected by centrifugation, washed with H_2O (again centrifuged), dried *in vacuo*, and extracted with $CHCl_3$-MeOH (1:1). The solution was evaporated to dryness *in vacuo*, weighed and examined by TLC. Satisfactory results were obtained by incubation of 0.5~2% Ex solution with twice~one-fourth amount of any enzyme used (except for hemicellulase) as much as that of Ex for 4 days. Some examples are as follows: Ex (gitogenin glycoside: ca. 60%) (TLC, Fig. 2c), 2 g.; H_2O, 200 ml.; "takadiastase-B," 0.5 g., 1 g., 2 g., 4 g.; at 37° for 4 days; yield of $CHCl_3$-MeOH extract (TLC, Fig. 2d), 0.54 g., 0.52 g., 0.5 g., 0.48 g., respectively. In larger scale experiments, above Ex (40 g.) and "takadiastase-B" (10 g.) in H_2O (4 L.) at 37° for 4 days gave FSE (10.6 g.) as a crystalline solid (recrystallized from CH_2Cl_2-MeOH) (TLC, Fig. 2e), while another Ex (gitogenin glycoside: ca. 85%) (30 g.) and "takadiastase-B" (60 g.) afforded FSE (12 g.) as needles (recrystallized from $BuOH-H_2O$).

Preparation of F-Gitonin and Desgalactotigonin——FSE thus obtained from the crude saponin or from the BuOH extract was separated into pure F-gitonin and desgalactotigonin in a larger scale by silica gel chromatography as described for that from I.

In the case when FSE contained ca. 85% F-gitonin, the sample (9.2 g.) was recrystallized from 70% EtOH and then from $BuOH-H_2O$ to give F-gitonin (6.2 g.) of ca. 94% purity[*8] as fine needles, m.p. 246~252° (decomp.), and further recrystallization (8 times) from $BuOH-H_2O$ afforded that of almost 100% purity as fine needles, m.p. 243~252° (decomp.). However, in another sample of FSE (F-gitonin: ca. 60%) of which solubility in $BuOH-H_2O$ was relatively high, it was hard to obtain pure F-gitonin only by recrystallization. Thus, the sample (500 mg.) gave a crystalline powder (200 mg.) (F-gitonin: ca. 60%), m.p. 256~258° (decomp.) (from dil. MeOH and MeOH or CH_2Cl_2-MeOH), or needles (120 mg.) (F-gitonin: ca. 70%), m.p. 256~260° (decomp.) (from $BuOH-H_2O$ (twice)).

The authors thank Shionogi & Co., Ltd. for the butanol extracts of the digitalis leaves, Prof. T. Momose and Dr. Mukai for the kind advices and helps in the micro determination of sugars, and Miss N. Nasu for technical assistance.

[*10] Commercial preparation (Sankyo Co., Ltd.).

[*11] Commercial preparation (Meiji Seika Co., Ltd.). An enzyme mixture (α- and β-amylases (main) and protease, lypase, cellulase, etc.) from *Aspergillus niger*. Kindly furnished by Dr. S. Kawaji of Meiji Seika Co., Ltd., to whom the authors are grateful.

[*12] Commercial preparation (Meiji Seika Co., Ltd.). An enzyme mixture (cellulase (main) and cellobiase, xylanase, amylase, etc.) from *Trichoderma koningi*. Kindly furnished by Dr. S. Kawaji of Meiji Seika Co., Ltd., to whom the authors are grateful.