17-H-20-one steroids to complete equilibrium mixture of the side chain in most cases.[6,11] The above facts strongly suggest that pergularin (Ⅵ) has C/D *cis* ring juncture, and 17β-OH, 17α-$COCH_3$ side chain.[6] Since sarcostin (Ⅰ), and four other compounds (Ⅱ, Ⅲ, Ⅳ, Ⅴ), which have 3β,12β,14β-OH groups, have been isolated from the same plants, biogenetic analogy would favour the structure (Ⅵ) for pergularin. This assumption was proved by the following results. Pergularin (Ⅵ) was reduced with $NaBH_4$, and the product examined by paper chromatography ($CHCl_3$/formamide),[8] giving two spots. The major spot was identical with that of utendin (Ⅴ). On partition chromatography over Celite(C_6H_6+BuOH/H_2O), crystals, m.p. 240~250°, were isolated, which was identified with utendin (Ⅴ) by a mixed fusion. Utendin has been isolated from *Pacycarpus lineolatus*,[10] 5α-dihydroutendin=tomentogenin (Ⅸ) from *Marsdenia tomentosa*.[11] Recently, the structures of these compounds (Ⅴ, Ⅸ) were established by Reichstein's group[12] and Mitsuhashi's group,[13] independently.

Thus perugularin is represented by the structure (Ⅵ).

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9) M. B. Rubin : Steroids, **2**, 561 (1963).
10) E. Abish, Ch. Tamm, T. Reichstein : Helv. Chim. Acta, **42**, 1014 (1959).
11) H. Mitsuhashi, I. Takemori, Y. Shimizu T. Nomura, E. Yamada : This Bulletin, **10**, 804 (1962).
13) A. Lardon, W. Klyne, E. Iseli, T. Reichstein : IUPAC Symposium at Kyoto, Japan, On April 18, 1964.
13) H. Mitsuhashi, T. Sato, T. Nomura, I. Takemori : This Bulletin, **12**, 981 (1964).

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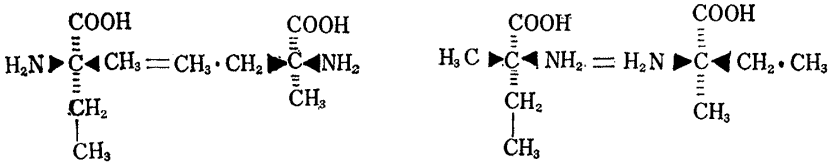
The Absolute Configuration of Optically Active Isovaline

Many of the recent studies on α-alkyl-α-amino acids have revealed that some of them have physiologically very interesting properties,[1] and few were found in natural products.[2] The absolute configuration of these amino acids is either still unknown or just only suggestive.[3~5]



$S^{a)}$-isovaline IIa IIb

$R^{a)}$-isovaline IIIa IIIb

a) cf. R.S. Cahn, C.K. Ingold, V. Prelog : Experientia, **12**, 81 (1956).

1) *a*) L. Gillespie, Jr. : Ann. N.Y. Acad. Sci., 88, 1011 (1960). *b*) H. Smirk : Brit. Med. J., **1963**, 146 and references therein. *c*) A. Sjoerdsma, S. Udenfriend : Biochem. Pharmacol., **8**, 164 (1961).
2) *a*) E.H. Flynn, J.W. Hinman, E.L. Caron, D.O. Woolf, Jr. : J. Am. Chem. Soc., **75**, 5867 (1953). *b*) Sheng-din Fang, Liang-chüan Li, Ching-i Niu, Kwong-fong Tseng : Sientia Sinica, **10**, 845 (1961). *c*) Tsun-tsi Sun, Shuen-hsing Loh, Shu-wei Chow. Zu-yoong Kyi : Scientia Sinica, **10**, 852 (1961). *d*) G.W. Kenner, R.C. Sheppard : Nature, **181**, 48 (1958). *e*) M. Kandatsu, K. Kikuno : Agr. Biol. Chem. (Japan), **25**, 234 (1961).

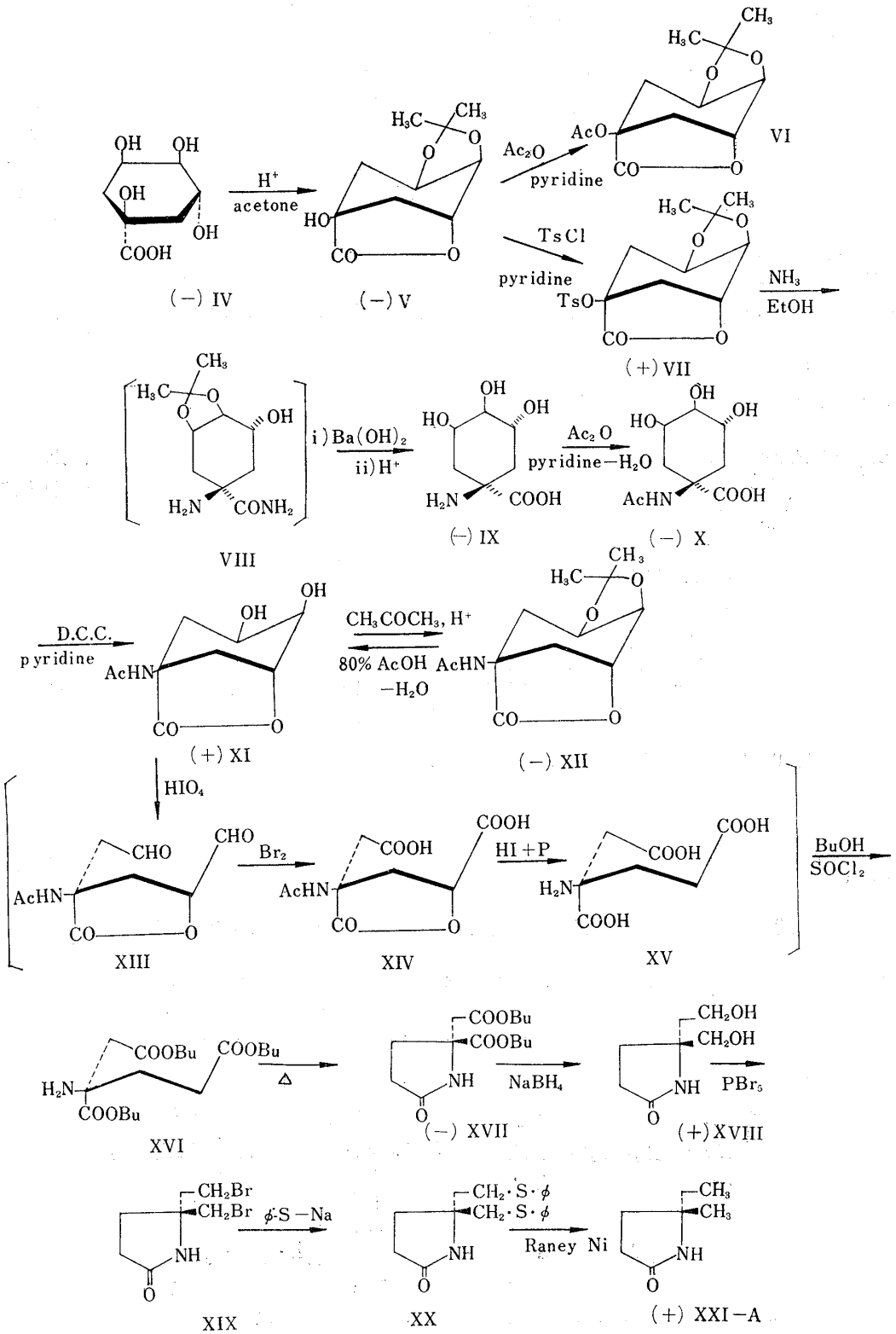


Chart 1.

3) E. W. Tristram, J. Ten Broeke, D. F. Reihold, M. Sletzinger, D. E. Williams : J. Org. Chem., **29**, 2053 (1964).
4) J. P. Greenstein, in M. L. Anson, K. Bailey, J. T. Edsall (Editors) : "Advances in protein chemistry," Vol. 9, Academic press, Inc., New York, 1954. p. 185.
5) E. M. Wilson, E. E. Snell : J. Biol. Chem., **237**, 3180 (1962).

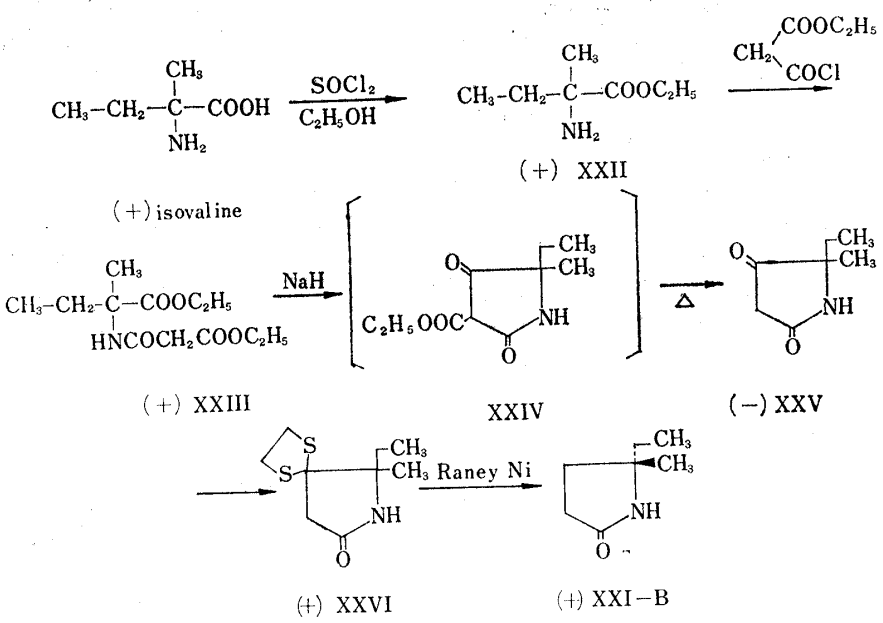


Chart 2.

The absolute configuration of (+)isovaline, the simplest and well known optically active α-methyl-α-amino acid, have been also proposed as the L-series from its taste[6] and its hydrolytic specificity to the action of hog kidny acylase.[7] The later assignment was based on the fact that the hydrolysis of N-chloroacetyl -DL-isovaline by hog kidny acylase yielded (+)isovaline, and hence this was assigned to L-isovaline (IIa). However a single isomer of isovaline may be considered to belong to either L- or D-series of amino acids depending upon the type of compound whether it is derived from L-butyrine (IIa) or D-alanine (IIb) in its origin, and the antipode is also considered as a derivative of D-butyrine (IIIa) or of L-alanine (IIIb).

In assigning the configuration of L-isovaline (IIa) to (+)isovaline, the assumption was thus made that the hydrolytic specificity of hog kidny acylase toward N-acyl-DL-isovaline was governed mainly by the butyrine skeletone and not by the alanine skeletone of this compound. It may only be said that (+) isovaline exhibits the same metabolic susceptibility as the naturally occurring L-amino acids, however there still remains a considerable doubt about the shown absolute configuration of (+) isovaline.[7b] Moreover, an aqueous solution of, what is called, "L-(+)isovaline" showed a small negative shift in optical rotation on addition of acid.[8] This phenomenon is certainly contrary to Clough-Lutz-Jirgensons rule applicable to naturally occurring L-amino acids. The absolute configuration of (+)isovaline is thus uncertain.

In the present communication, we wish to report the chemical correlation between optically active isovaline and the (−)quinic acid, whose absolute configuration was clearly established,[9] aiming to determine the absolute cofiguration of isovaline.

The isopropylidene lactone (V), prepared from D-(−)quinic acid (IV) (m.p. 164~166° $[\alpha]_D^{31}$ −45.1 (c=0.328, H_2O)) according to the method of Eberle, *et al.*,[10] was tosylated to

6) T. Kaneko: Nippon Kagaku Kaishi, **60**, 531 (1939).
7) *a*) C. G. Baker, S-C. J. Fu, S. M. Birnbaum, H. A. Sober, J. P. Greenstein: J. Am. Chem. Soc., **74**, 4701 (1952). *b*) J. P. Greenstein, M. Winitz: "Chemistry of the amino acids," Vol. 1, John Wiley and Sons, New York, 1961, p. 85, 748.
8) M. Winitz, S. M. Birnbaum, J. P. Greenstein: J. Am. Chem. Soc., **77**, 716 (1955).
9) G. Dangschat, H. O. L. Fischer: Biochim. et Biophys. Acta, **4**, 199 (1950) and references therein.
10) M. Eberle, D. Arigoni: Helv. Chim. Acta, **43**, 1508 (1960).

O-tosylate (Ⅶ), m.p. 96~97°,*1 $[\alpha]_D^{16}$ +23.1 (c=2.54, benzene). IR ν_{max}^{KBr} cm^{-1} : 1809 (5-membered lactone), 1352, 1174 (-SO_3R). The reaction with alcoholic ammonia, followed by the hydrolysis of Ⅶ with 10% barium hydroxide afforded the amino acid (Ⅸ), monohydrate : m.p. 260~261° (decomp.), $[\alpha]_D^{24}$ −65.0 (c=0.50, H_2O), IR ν_{max}^{KBr} cm^{-1} : 3469 (broad) (ν_{O-H}), 2915 (broad)($\nu_{NH_3^+}$), 1657 ($\delta_{NH_3^+}$), 1601 (COO^-), 1077 (broad).

Two diastereoisomers, Ⅸ and Ⅸa, should be considered to this product, but the product obtained, was demonstrated to have the structure (Ⅸ) by nuclear magnetic resonance spectroscopy. Even though the amino acid would be represented as Ⅸa, Ⅸa would be led to the same derivatives (XIII) *via* Xa and XIa as it is derived from Ⅸ by the similar reaction sequences. For the present purpose, it is not important whether the amino acid is represented by Ⅸ or Ⅸa. (Chart 3).

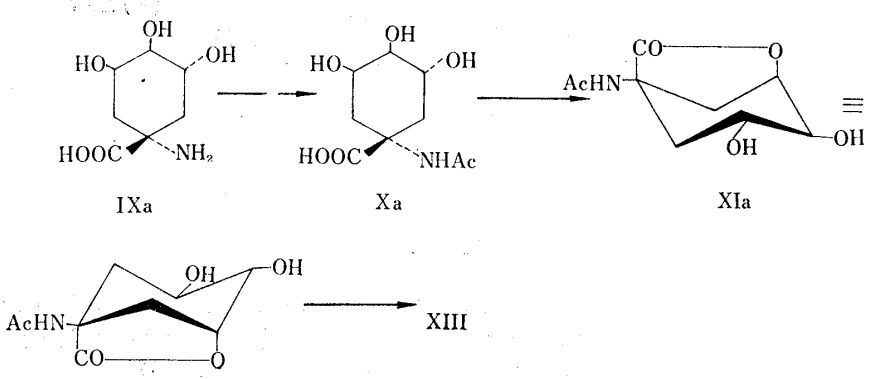


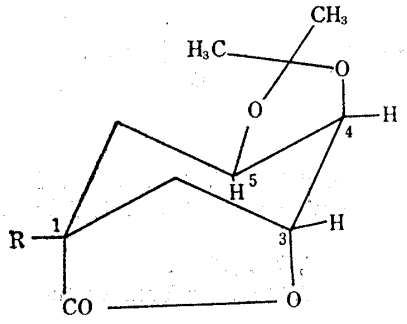
Chart 3.

Ⅸ was acetylated by actyl anhydride in aqueous pyridine to yield N-acetyl derivative (Ⅹ), m.p. 193~194°, $[\alpha]_D^{29}$ −92.3 (c=0.234, CH_3OH). IR ν_{max}^{KBr} cm^{-1} : 3469, 3394 (ν_{O-H}), 3270 (ν_{N-H}), 2800~2500, 1707 (COOH), 1638, 1533 (amide), 1072, 1053. Ⅹ was lactonized with equimolecular N,N-dicyclohexylcarbodiimide to give the diol-lactone (Ⅺ), m.p. 206~207.5 (decomp.), $[\alpha]_D^{19}$ +5.3 (c=0.524, CH_3OH). IR ν_{max}^{KBr} cm^{-1} : 3536, 3407, 3224 (ν_{O-H}, ν_{CONH-}), 1788 (5-membered lactone), 1661, 1533 (amide). Reaction of Ⅺ with acetone-sulfuric acid afforded the isopropylidene-lactone (Ⅻ), m.p. 198~199°, $[\alpha]_D^{30}$ −14.7 (c=0.19, CH_3OH). IR ν_{max}^{KBr} cm^{-1} : 3367 (ν_{N-H}), 1780 (5-membered lactone), 1684, 1537 (amide). Hydrolysis of Ⅻ with 80% acetic acid gave Ⅺ. By comparing nuclear magnetic resonance spectrum of Ⅻ with the stereochemically well established Ⅵ,[11] the conformation of Ⅺ was assigned as shown, therefore the conformation of the above (Ⅸ) was evidently established.[12] Periodate oxidation of Ⅺ yielded the dialdehyde (XIII) which was oxidized by bromine to dicarboxylic acid (XIV). XIV was reduced with red P and hydroiodic acid to the tricarboxylic acid

*1 All analytical data of the compounds shown by melting point or boiling point in this report were identical with theoritical data.

11) K. Josephson : Ber., **61**, 911 (1928).

12)

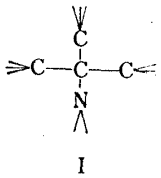


Ⅵ (R=AcO) : $J_{H_3-H_4}$ 2.5 c.p.s., $J_{H_4-H_5}$ 6.4 c.p.s.
Ⅻ (R=AcNH) : $J_{H_3-H_4}$ 2.3 c.p.s., $J_{H_4-H_5}$ 5.7 c.p.s.
Therefore C_4-H of Ⅻ is conformationally equatorial as same as the C_4-H of Ⅵ.

(XV), which was esterified with butanol and thionyl chloride. The ester (XVI) was lactamized under vacuum distillation to yield the lactam-ester (XVII), b.$p_{4.5}$ 199~201°, $[\alpha]_D^{18}$ −27.4 (c=0.576, benzene). IR ν_{max}^{Film} cm^{-1} : 3460, 3270 (ν_{N-H}), 1749, 1742 (ester), 1718 (5-membered lactam). Reduction of XVII with sodium borohydride in alcohol afforded the diol (XVIII), oil, $[\alpha]_D^{26}$ +8.8° (c=1.90, CH_3OH). IR ν_{max}^{Film} cm^{-1} : 3329 (ν_{O-H}), 1674 (5-membered lactam). Di-*p*-nitrobenzoate, m.p. 191~192°. Bromination of XVIII, followed by thioetherification with thiophenol, and desulfurization by Raney nickel yielded the lactam, 5-ethyl-5-methyl-2-pyrrolidinone (XXI)-(A), b.$p_{15.5}$ 135~138°, $[\alpha]_D^{28}$ +8.9 (c=0.56, benzene). IR ν_{max}^{Film} cm^{-1} : 3436, 3264 (ν_{N-H}), 1695 (5-membered lactam). (Chart 1).

On the other hand, lactam (XXI) was synthesized from (+)isovaline (monohydrate: $[\alpha]_D^{28}$ +6.7° (c=1.86, H_2O)) as shown in Chart 2. Esterification of (+) isovaline with alcohol and thionyl chloride yielded the isovaline ethyl ester (XXII). b.$p_{25\sim30}$ 75~80°, $[\alpha]_D^{30}$ +1.5° (c=1.75, benzene). IR ν_{max}^{Film} cm^{-1} : 3379, 3342 (ν_{N-H}), 1730 (ester). Picrate m.p. 145~146°. This ester (XXII) was submitted to Schotten-Baumann reaction with malonic half ester chloride in the presence of potassium carbonate in acetone to yield the amide ester (XXIII), b.$p_{5.5}$ 154~156°. $[\alpha]_D^{33}$ +4.6 (c=2.096, benzene). IR ν_{max}^{Film} cm^{-1} : 3360 (ν_{N-H}), 1740 (ester), 1658, 1548 (amide). This amide ester underwent the Dieckmann cyclization with sodium hydride in dioxane to afford keto-ester (XXIV), which was readily decarboxylated to give the keto-lactam (XXV), m.p. 105~106°, $[\alpha]_D^{29}$ −11.9° (c=0.115, CH_3OH). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3411, 3238 (ν_{N-H}), 1771 (5-membered ketone), 1702 (5-membered lactam). Ketalization of XXV with ethylene thioglycol in the presence of boron trifluoride etherate gave the thioketal (XXVI), m.p. 171~171.5°, $[\alpha]_D^{29}$ +16.1° (c=0.31, CH_3OH). IR ν_{max}^{KBr} cm^{-1} : 3173, 3053 (ν_{N-H}), 1712 (5-membered lactam). This thioketal (XXVI) was desulfurized with Raney nickel to give the lactam, 5-ethyl-5-methyl-2-pyrrolidinone (XXI)-(B), b.$p_{11.5}$ 130~135°, $[\alpha]_D^{29}$ +5.7° (c=0.848, benzene). IR ν_{max}^{Film} cm^{-1} : 3436, 3262 (ν_{N-H}), 1695 (5-membered lactam).

Two lactams (XXI) (A and B) which were obtained by the different way from D-(−)-quinic acid and (+)isovaline were identical in all respects, infrared spectra, optical rotatory curves and retention times of gas chromatography. Accordingly, the absolute configuration of (+)isovaline was obviously determined to be either (IIa) or (IIb), and this is the first report to determine the absolute configuration of tertiary carbinamine skeleton (I) by direct correlation of glyceraldehyde.



I

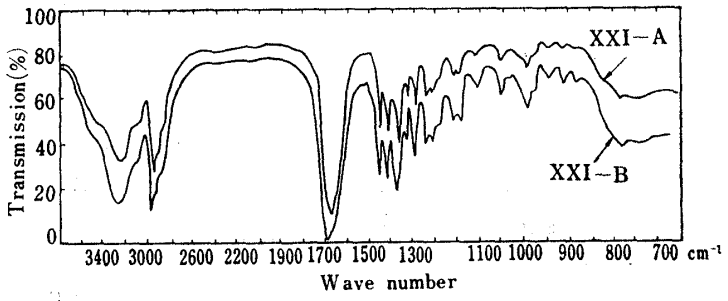


Fig. 1. Infrared Spectra of XXI-A and XXI-B

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