granules, mp 232–233° (decomp.) (EtOH), Rf 0.72 ($CHCl_3$: EtOH, 1:1). (Found[5]: C, 51.65, 51.50; H, 8.44, 8.32.) Saponin C, when crystallized from 80% alcohol, yielded fine needles of Saponin D, mp 301–302° (decomp.), Rf 0.72 ($CHCl_3$:EtOH, 1:1). (Found[5]: C, 56.87, 57.06; H, 8.83, 8.68.)

Both saponins C and D yielded on acid hydrolysis diosgenin, glucose and rhamnose. The saponins B, C and D differ from each other probably in their contents of the relative proportion of glucose and rhamnose, the former always predominating. All the 3 saponins had a direct spasmodic activity on isolated uterus.

Free diosgenin along with free tigogenin was also isolated from the petroleum ether extract of the dried powdered rhizomes. The sapogenins at every stage were characterized by comparison with authentic specimens[6] from a study of their TLC pattern, mp, mixed mp, specific rotation, analysis[4], IR-, NMR- and mass-spectra[7].

*Zusammenfassung.* Eine neue, ergiebige Quelle zur Gewinnung von Diosgenin aus *Costus speciosus* wird beschrieben.

B. DASGUPTA and V. B. PANDEY

*Department of Medicinal Chemistry,*
*Research and Post Graduate Institute of Indian Medicine,*
*Banaras Hindu University,*
*Varanasi-5 (India), 19 November 1969.*

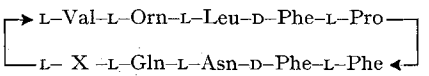
[5] Microanalysis was done by Central Drug Research Institute, Lucknow (India).

[6] Grateful thanks are due to Dr. Y. SATO, Section of Steroids, NIAMD, National Institute of Health, Bethesda (Maryland, USA), for supply of authentic samples of tigogenin, diosgenin, sarsasapogenin and smilagenin.

[7] IR-, NMR- and mass-spectra were determined by the National Chemical Laboratory, Poona (India).

## Synthesis of a Cyclic Decapeptide Corresponding to Tyrocidine E

Tyrocidine E (TE) is a new basic polypeptide isolated in 1968 by KURAHASHI et al.[1] from an incubation mixture in a cell-free enzyme system of *Bacillus brevis* in the absence of both tyrosine and tryptophan. They have suggested the structure of TE as a cyclic decapeptide shown as XI[1] by means of comparison of the amino acid composition of TE sample with that of tyrocidine A (TA) since the amino acid sequence of TA has been already established[2,3]. However, they did not describe any of the physical and biological properties of the peptide.




Structure of tyrocidine E and A. X represents an amino acid residue such as Phe (TE or XI) and Tyr (TA).

We reported previously the synthesis of tyrocidine A[3] and B[4], and have been attempting to synthesize other tyrocidines. We wish to report here the synthesis of the cyclic decapeptide (XI) designated as TE, and the chemical and the biological properties of the synthetic product.

Condensation of Z-Gln-ONp[5] with H-Phe-OEt gave Z-Gln-Phe-OEt (I), mp 167–169°, $[\alpha]_D$ − 4.0°, which was hydrogenated with an equivalent of hydrogen chloride to produce H-Gln-Phe-OEt·HCl (II), mp 178–182°, $[\alpha]_D$ +17.0°. Z-Asn-Gln-Phe-OEt (III), mp 225–227°, $[\alpha]_D$ −16.0°, obtained from Z-Asn-ONp and II, was also converted to H-Asn-Gln-Phe-OEt·HCl (IV) by hydrogenation, mp 199–202°, $[\alpha]_D$ −8.0°. Z(OMe)-Phe-D-Phe-Asn-Gln-Phe-OEt (V), mp 212–215°, $[\alpha]_D$ −16.4°, was obtained by condensation of IV and the azide derived from Z(OMe)-Phe-D-Phe-$NHNH_2$[3]. V was treated with hydrazine to afford Z(OMe)-Phe-D-Phe-Asn-Gln-Phe-$NHNH_2$ (VI), mp 218–221°, $[\alpha]_D$ −23.5° (dimethylsulfoxide). Condensation of the azide derived from VI with H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH[3] gave Z(OMe)-Phe-D-Phe-Asn-Gln-Phe-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH (VII), mp 236–239°, $[\alpha]$ −31.8° (dimethylsulfoxide). Treatment of VII with *di-p*-nitrophenyl sulfite gave amorphous acyldecapeptide *p*-nitrophenyl ester (VIII). The Z(OMe) group of VIII was removed by the action of trifluoroacetic acid and the decapeptide *p*-nitrophenyl ester trifluoroacetate obtained was treated with hot pyridine for the cyclization reaction[6]. Purification of the crude product by passing its aqueous dioxane-methanol solution through columns of Dowex 50 ($H^+$ form) and Dowex 1 ($OH^-$ form) gave cyclo-Phe-D-Phe-Asn-Gln-Phe-Val-Orn(δ-Z)-Leu-D-Phe-Pro (IX), yield 40% (from VII), mp 252–255° dec, $[\alpha]_D$ −129° (methanol) (mol. wt. calcd. for $C_{74}H_{93}O_{14}N_{13}\cdot 2H_2O$: 1425; found: 1435).

Removal of the Z group from IX by hydrogenation in the presence of an equivalent of hydrogen chloride in methanol provided crystalline cyclo-Phe-D-Phe-Asn-Gln-Phe-Val-Orn-Leu-D-Phe-Pro·HCl·$3H_2O$ (XI·HCl·$3H_2O$) as a desiccator-dried product, 84%, mp 265–267° dec, $[\alpha]_D$ −126° (methanol), amino acid ratios in acid hydrolysate; Phe 3.9, Asp 1.0, Glu 1.0, Val 0.9, Orn 1.0, Leu 1.0, Pro 1.0, $NH_3$ 1.9. Its homogeneity was ascertained by thin-layer and paper chromatographies, and paper electrophoresis with pH 1.8 buffer (Rf 0.66 × gramicidin S). The antibacterial activity of synthetic XI was determined by a dilution method with a bouillon agar medium and with a synthetic medium both at pH 7.0, that of TA as a reference peptide being also determined under same condition. It

[1] K. FUJIKAWA, Y. SAKAMOTO, T. SUZUKI and K. KURAHASHI, Biochim. biophys. Acta *169*, 520 (1968).

[2] A. R. BATTERSBY and L. C. CRAIG, J. Am. chem. Soc. *74*, 4019 (1952).

[3] M. OHNO and N. IZUMIYA, J. Am. chem. Soc. *88*, 376 (1966). – M. OHNO, T. KATO, S. MAKISUMI and N. IZUMIYA, Bull. chem. Soc. Japan *39*, 1738 (1966).

[4] K. KUROMIZU and N. IZUMIYA, will be presented at the 7th Symposium on Peptide Chemistry at the University of Tokyo, Tokyo, 21 November 1969; Experientia, in preparation.

[5] Satisfactory elemental analyses and chromatographic data were obtained for all crystalline compounds described here. $[\alpha]_D$ refers to a solution in dimethylformamide at 15° otherwise noted. Z, benzyloxycarbonyl; Z(OMe), *p*-methoxybenzyloxycarbonyl; ONp, *p*-nitrophenoxy. Amino acid symbols except D-Phe denote the L configuration.

[6] R. SCHWYZER and P. SIEBER, Helv. chim. Acta *40*, 624 (1957).

was found that the degree of the activities of XI toward Gram positive microorganisms was nearly the same to that of TA; with the synthetic medium, minimum concentrations of growth-inhibition for *Bacillus subtilis* are 5 μg/ml for XI and 8 μg/ml for TA, and that for *Staphylococcus aureus* are 10 μg/ml for XI and 8 μg/ml for TA. The results indicates that the L-tyrosine residue in TA can be replaced by L-phenylalanine without an influence for the activity.

*Zusammenfassung.* Die Synthese des dem Tyrocidin E entsprechenden, zyklischen Dekapeptids, eines bei der Zyklisierung des aktiven Esters linearen Dekapeptids, wird beschrieben.

N. MITSUYASU and N. IZUMIYA

*Laboratory of Biochemistry, Faculty of Science, Kyushu University, Fukuoka (Japan), 17 November 1969.*

## Chemical Investigation of *Abroma augusta* Linn. Identity of Abromine with Betaine

*Abroma augusta* Linn. (*Ulatkambal*, N.O. Sterculiaceae), a small tree growing wild in India, is a popular medicine in the indigenous systems. The root and root-bark are reputed remedies as an emmenagogue for congestive and nervous dysmenorrhoea, and the leaves and stems are reported to be very efficacious in gonorrhoea. The root was reported to contain an alkaloid, abromine[1], $C_6H_{13}NO_2$, mp 283–285°; a sterol, $C_{30}H_{52}O_2$, mp 153–157°; friedelin[2] and abromasterol A, mp 125.5°. A recent short communication[3] reporting the isolation of taraxeryl acetate, taraxerol, β-sitosterol and a low melting neutral compound from the petroleum ether extract of the leaves, prompts us to report here the chemical work[13] we have been carrying out on the roots and leaves of this plant to explain its pharmacodynamic activity[4,5] and to characterize the alkaloid, abromine, and the sterols isolated previously.

The quaternary bases isolated as reineckates by the procedure followed in *Pluchea lanceolata*[6], were found to contain choline, betaine and a base yielding a picrate, mp 223–227°[7]. The method of isolation of abromine[1] and the reported mp of its derivatives indicate beyond doubt its identity with betaine in view of our isolation of the latter from the roots. The non-volatile, non-saponifiable fraction of the petroleum ether extract of the roots on chromatography over aluminium oxide yielded 2 sterols giving a violet to green colour (through blue) with Liebermann-Burchard reagent. These were identified as β-sitosterol (m/e 414 $M^+$) and stigmasterol (m/e 412 $M^+$).

The petroleum ether extract of the leaves of *A. augusta* on similar treatment yielded the following 5 compounds having different Rf values as revealed by thin-layer chromatography ($SiO_2$; $C_6H_6$:$CHCl_3$, 1:1; $I_2$ vapour or $AC_2O$–$H_2SO_4$–EtOH mixture as developer):

1. Compound A from petroleum ether eluant, granular solid, mp 84–85° (EtOAC), Rf 0.35 ($I_2$ vapour) identical spot with octacosanol, freely soluble in pet. ether, $C_6H_6$, and gave no colouration with Liebermann-Burchard reagent. Found[8]: C, 81.42, 81.30; H, 14.22, 14.10. $C_{28}H_{58}O$ requires: C, 81.87; H, 14.23. IR[9]-absorption peaks at 3220 (OH), 2860, 1465, 1400, 1380, 1125, 1075, 1065, 1020, 735 and 722 [$-(CH_2)_n$-rocking split] $cm^{-1}$ in Nujol, which compared favourably well with those of octacosanol. NMR-spectrum in $CHCl_3$ showed a prominent peak at 1.25 δ due to methylene protons and small peaks at 0.9 δ and 3.65 δ. The mass spectrum exhibited prominent higher mass peaks at m/e 392 (M-18) and 422 (M'-28) with smaller mass peaks at m/e 450, 451, 436, 423, 408, 407, 394, 393, 378, etc. The general fragmentation pattern of the mass spectrum indicated[10] the compound A to be a mixture of octacosanol, $C_{28}H_{58}O$ (M-410) and the alkane, $C_{32}H_{66}$ (M'-450), the former predominating.

2. Compound B from pet. ether eluant, fine needles, mp 260–268° (pet. ether), Rf 0.95, soluble in pet. ether, $C_6H_6$, $CHCl_3$, gave a pink colour with Liebermann-Burchard reagent. IR-spectrum was very similar to that of compound C with additional absorptions at 3250 $cm^{-1}$ (primary OH) and 722 and 735 $cm^{-1}$ [$-(CH_2)_n$-rocking split]. NMR-spectrum in $CHCl_3$ showed a prominent methylene proton peak at 1.25 δ and methyl proton peaks at 0.82, 0.95, 1.1 and 1.6 δ. A comparative study of the IR-, NMR- and mass-spectra indicated the compound to be a mixture of taraxerol[11] [m/e 426 (M), 411 (M-$CH_3$), 302 (K), 287 (K'), 284 (K-$H_2O$), 204 (1), 189 (1-$CH_3$) and an aliphatic alcohol[10], $C_{32}H_{66}O$ [m/e 448 (M-18), 420 (M-18-28), 392 (M-18-2×28)].

3. Compound C from pet.ether:$C_6H_6$ (1:1) eluant, crystalline rods, mp 279–280° ($C_6H_6$), Rf 0.91, sparingly soluble in $C_6H_6$, $CHCl_3$, insoluble in pet. ether, soluble in acetone, ethyl acetate and alcohol and gave a pink colour with Liebermann-Burchard reagent. The mass-spectrum exhibited the usual fragmentation pattern of taraxerol[11]. The identity of the compound with taraxerol was confirmed by comparison of mp, mixed mp of the alcohol and its acetate and IR-spectrum with those of an authentic sample[12].

4. Compound D from $C_6H_6$ and $CHCl_3$ eluants, needles, mp 134–135° (alcohol), Rf 0.28, gave a violet to blue to green colour with Liebermann-Burchard reagent, formed an acetate, mp 127–128° which did not show any depression in mixed mp with the acetate of β-sitosterol.

[1] G. P. SRIVASTAVA and N. K. BASU, Ind. J. Pharmacy. *18*, 472 (1956).
[2] S. ALI, A. M. AHSAN and G. HANN, Pakist. J. scient. ind. Res. *1*, 305 (1958).
[3] N. ADITYACHAUDHURY and P. K. GUPTA, J. Ind. chem. Soc. *46*, 849 (1969).
[4] S. K. BHATTACHARYA, R. LAL and P. K. DAS, Ind. J. Pharmac. *1*, 7 (1969).
[5] S. K. BHATTACHARYA, R. LAL, K. BASU and P. K. DAS, J. Res. ind. Med., 4, 176 (1970).
[6] B. DASGUPTA, Experientia *23*, 989 (1967).
[7] B. DASGUPTA, K. BASU and S. DASGUPTA, Experientia *24*, 882 (1968).
[8] Microanalyses were done by Dr. F. B. STRAUSS, Microanalytical Laboratory, Oxford (England) and Central Drug Research Institute, Lucknow (India).
[9] All IR-, NMR- and mass-spectra were scanned by the National Chemical Laboratory, Poona (India).
[10] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Interpretation of Mass Spectra of Organic Compounds* (Holden-Day, San Francisco 1964), p. 32.
[11] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, J. Am. chem. Soc. *85*, 3688 (1963).
[12] Grateful thanks are due to Prof. L. R. Row, Andra University, Waltair (India), for supply of authentic samples of taraxerol and its acetate with IR-absorption curves.