IR 1760 (lactone), 1620, 1590 (aromatic), 1135 (C–O–C) $cm^{-1}$, NMR $\delta$ ppm ($CDCl_3$), 6.99, 6.88 (1H, each, due to H-5 and H-6, J = 8Hz), 2.8 (2H, each, m, due to H-3, H-4 and H-10), 1.78 (2H, t, due to H-9, J = 7Hz), 1.32 (6H, s, gem dimethyl), found: C, 71.96; H, 6.96. $C_{14}H_{16}O_3$ requires: C, 72.39; H, 6.94%]. The compound II was dehydrogenated with Pd-C (10%) in boiling diphenyl ether to give dihydroseselin [III, mp and mmp[10] 102–103° (lit.[11] mp 103–104°), IR 1730 ($\alpha\beta$-unsaturated lactone), 1600, 1490 (aromatic), 1120 (C–O–C), $cm^{-1}$ (Nujol), UV $\lambda_{max}^{EtOH}$ 222, 247, 258, 328 (log $\varepsilon$ 4.1, 3.6, 3.5 and 4.3), NMR $\delta$ ppm ($CDCl_3$) 7.60, 6.20 (1H each, d, due to H-4 and H-3, J = 10Hz), 7.20, 6.70 (1H each, d, due to H-5 and H-6, J = 9Hz), 2.90, 1.85 (2H each, t, due to H-9 and H-10), 1.36 (6H, s, gem dimethyl), found: C, 73.40; H, 6.07. $C_{14}H_{14}O_3$ requires: C, 73.02; H, 6.13%]. Dihydroseselin (III) on further dehydrogenation with DDQ[12] in dioxan yielded seselin (IV) mp 113–114° (depression in mp with dihydroseselin). Found: C, 73.42; H, 5.70. $C_{14}H_{12}O_3$ requires: C, 73.67; H, 5.30%. Further work is in progress and more details will be published in due course[13].

*Zusammenfassung.* Die Synthese des Naturstoffes Tetrahydroseselin aus der Frucht *Seseli indicum* wird beschrieben.

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## A New Indian Source of Diosgenin (*Costus speciosus*)

In an extensive survey of 19 different species of Indian *Dioscorea* plants, Chakravarti et al.[1] found *Dioscorea prazeri* (*Kukur torul*) of Darjeeling and *Dioscorea deltoidea* (*Kins*) of Kashmir to be 2 rich sources of diosgenin. The yield of diosgenin was 2.1% from the former and 3.35% from the latter on the basis of dried yams. We have recently found a new source of diosgenin in the rhizomes of *Costus speciosus* which contains about 86.3% moisture. The alcoholic extract of the dried rhizomes on acid hydrolysis yields about 3.86% total sapogenins which on further crystallization and chromatography yields about 2.12% pure diosgenin (dry rhizomes). The diosgenin content of *Costus speciosus* compares favourably with that of *D. prazeri*, and can be utilized as a convenient commercial source for isolation of diosgenin. The main advantage of *C. speciosus* over the 2 species of *Dioscorea* is that *C. speciosus* grows abundantly in the plains, whereas both *D. prazeri* and *D. deltoidea* grow only at high altitudes of the Himalayas.

*Costus speciosus* (Koenig) Sm (N.O. Zingiberacea) (Sanskrit: *Kemuka*)[2] is a common plant with tuberous rhizome distributed throughout India, particularly in Bengal and Konkan. From a pharmacological study, Tewari et al.[3] reported that the fresh juice of the rhizomes of *C. speciosus* increased the tone, amplitude and frequency of rhythmic contractions of isolated uterus of rat, guinea-pig, rabbit, dog and human. The spasmodic activity was not blocked by atropine sulphate and pentolinium bitartrate. In view of the above uterine activity of the plant and its use as an ecbolic in the indigenous systems of medicine, a detailed chemical investigation was undertaken to isolate the active principles.

A systematic chemical and pharmacological study indicated that the chloroform extract of dried powdered rhizomes, after being extracted with petroleum ether and benzene, possessed the most potent and direct stimulant action on isolated uterus. This extract was found to contain a mixture of 5 saponins of varying proportions as revealed by thin-layer chromatography (TLC). Partition chromatography over silica gel followed by repeated crystallization from alcohol could separate the mixture broadly into 2 groups of saponins. The earlier crops of saponins of higher Rf values (0.76 and 0.55, $CHCl_3$:EtOH, 7:3) had either relaxant action or no specific response, whereas the later crops of saponins of lower Rf values (0.20, 0.14 and 0.06, $CHCl_3$:EtOH, 7:3) had direct stimulant action on isolated uterus. On TLC plates the colour of the upper 2 saponins changed from pink to grey and that of the lower 3 saponins from pink to green on developing with an alcoholic solution of acetic anhydride and sulphuric acid, indicating the presence of 2 different types of saponins.

The following 2 saponins could be isolated from the chloroform extract of the dried powdered rhizomes of *C. speciosus*: Saponin A, granules, mp 289–290° (80% alcohol), Rf 0.71 ($CHCl_3$:EtOH, 7:3). (Found[4]: C, 72.02, 72.13; H, 10.09, 10.27.) On acid hydrolysis it gave glucose and $\beta$-sitosterol ($C_{35}H_{60}O_6$ requires: C, 72.91; H, 10.41), and had no specific response on isolated uterus.

Saponin B, granules, mp 305–307° (80% alcohol), Rf 0.43 ($CHCl_3$:EtOH, 1:1). (Found[5]: C, 58.72, 58.80; H, 9.02, 9.19.) On acid hydrolysis the saponin yielded diosgenin, glucose and rhamnose.

The alcoholic extract of the fresh moist rhizomes of *C. speciosus* yielded the following saponin: Saponin C,

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[4] Microanalysis was carried out by Dr. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford (England).

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.

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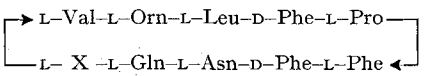
[5] Microanalysis was done by Central Drug Research Institute, Lucknow (India).

[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

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