and the control of exocrine and endocrine activities in pancreas cultures.

Work on lens regeneration has shown that neural retina is vital for lens regeneration from the iris. Extrinsic factors have also been cited as important in limb regeneration, and in view of the ease of testing such factors in vitro, it is surprising that so little work has been done on amphibian limb regenerates in organ culture.

Heterotypic interactions between epithelial and mesenchymal tissues are essential for the normal development of many organs (e.g. kidney [102]). One of the most important findings from research on induction in vitro was that there are mutual control systems between interacting tissues [103]. TARIN [104] has stressed the importance of heterotypic interactions in the maintenance of normal structure and function in the adult and their possible significance in carcinogenesis. We consider that amphibian organ cultures are of great potential importance in seeking solutions to these problems [105, 106].

Note added in proof. A number of articles have appeared since this review was written. CLEMENS, LOFTHOUSE and TATA [107] have repeated WALLACE and JARED'S [40] experiments on *X. l. laevis* liver, using serum-free, HEPES-buffered medium 199 with labelled amino acids. They found that liver explants from oestrogen-treated males secreted 4 times the amount of labelled protein as explants from untreated males, while there was no increase in the amount of radioactivity in tissue proteins over a 4-day period. Inhibitors of RNA synthesis inhibited the secretion of protein after 2–3 days. BALINSKY, COETZER and MATTHEYSE [108] cultured adult *X. l. laevis* liver cubes for up to 8 days in medium 199, and looked at the uptake of ^{3}H-leucine into carbamyl phosphate synthetase in animals which had been kept in normal or hypertonic saline. Animals kept in hypertonic saline excreted a higher proportion of their nitrogenous waste in the form of urea and had higher levels of carbamyl phosphate synthetase. Liver explants from such animals incorporated more ^{3}H-leucine into enzyme and experiments with puromycin showed that, unlike *R. catesbeiana* [65], no non-immunoprecipitable enzyme precursor was involved. MAHDAVI and CRIPPA [109] incubated ovaries from *X. l. laevis* tadpoles for 48 h in Leibovitz L-15 medium containing 10% foetal calf serum, ^{3}H-uridine and ^{14}C-thymidine to label the RNA-ribosomal DNA complex. HARPER and GROSS [110] have shown that *R. catesbeiana* tadpole tissues which produce collagenase in vitro, first secrete an inactive zymogen and later an activator, which converts the zymogen to active enzyme.

Résumé. Les auteurs passent en revue les problèmes étudiés et les méthodes utilisées jusqu'à ce jour pour la préparation des tissus des amphibiens en culture organotypiques. Ils envisagent en outre les possibilités qui s'offrent aux recherches futures.

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[106] *Appendix.* Full names of species listed in Tables I and II but not given in full in the text: *Bufo bufo bufo, Bufo marinus, Discoglossus picta, Hyla arborea savignji, Pleurodeles waltlii, Rana clamitans, Siredon mexicanum, Taricha torosa, Triturus cristatus.*
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SPECIALIA

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Arctigenin-4′-β-Gentiobioside from *Trachelospermum asiaticum* var. intermedium

In addition to the reported isolation of 4 lignan glucosides, arctiin, matairesinoside, tracheloside and nortracheloside from the stems of *Trachelospermum asiaticum* Nakai var. intermedium Nakai (Apocynaceae) [1–4], we now found a new lignan glycoside, arctigenin-4′-β-gentiobioside (I) the first example of a naturally occurring glucosyl glucoside of the lignan series.

The glycoside (I), a colorless crystalline powder, mp 174–176°, λ_{max}^{EtOH} nm (log ε) 230 (4.20), 280 (3.79), ν_{max}^{KBr} cm^{-1} 3400 (br. OH), 1770 (CO), 1595, 1515 (aromatic, C=C), $[\alpha]_D^{26}$ −57.2 (c = 1.0 in H_2O), Anal. Calcd. for $C_{33}H_{44}O_{16}\cdot H_2O$: C 55.46, H 6.49; Found: C 55.63, H 6.55, is obtained in 0.0004% yield from the chloroform-methanol (2:1 V/V) extractive of the residue after the extraction of four other lignan glucosides and gave heptaacetate, colorless needles, mp 183–184°, λ_{max}^{EtOH} nm (log ε) 229 (4.22), 279 (3.81), ν_{max}^{KBr} cm^{-1} 1760 (CO), 1595, 1515 (aromatic C=C), $[\alpha]_D^{21}$ −46.7 (c = 1.168 in $CHCl_3$), Anal. Calcd. for $C_{47}H_{58}O_{23}$:

C 56.97, H 5.90, mol. wt. 990.9; Found: C 57.07, H 6.06, mol. wt. 976.2 (vapor pressure osmometry in $CHCl_3$), the NMR-spectrum ($CDCl_3$) showed signals attributable to 3 aromatic methoxyls at δ 3.80(s), 3.85(s), seven aliphatic acetyls at δ 1.90(s), 2.05(s) and an anomer at δ 4.55 (d, J = 6 cps, β-linkage). Hydrolysis of I with 10% H_2SO_4 solution or emulsion gave D-glucose, which was detected by paper chromatography and gas-liquid chromatography as trimethylsilyl ether, and arctigenin, mp 94–95°, which was identified with an authentic sample by mixed melting point, mass and infrared spectral comparisons.

The permethyl ether prepared by the methylation of I with NaH, DMSO and CH_3I (HAKOMORI's method[5]) afforded on methanolysis with 3% methanolic hydrogen chloride methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside in almost equal ratio to those prepared from permethyl gentiobiose[6], which were detected by gas-liquid chromatography: condition: column temperature, 175°; carrier gas: N_2 (30 ml/min). On JEOL-JGC 1100 with flame ionization detector.

Therefore, the structure of I has been established to be 4′-*O*-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl) arctigenin (arctigenin-4′-β-gentiobioside).

Zusammenfassung. Eine neue Lignansubstanz, Arctigenin-4′-β-gentiobiosid $C_{33}H_{44}O_{16}\cdot H_2O$ wurde als weisses Pulver von Smp. 174–176° aus Stengeln von *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai gewonnen.

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Dehydrogenation of Khusilic Acid

In an earlier communication[1] a rare reduction of the carboxyl group of khusilic acid (I) to a methyl during selenium dehydrogenation to afford 1,6-dimethyl-4-ethyl naphthalene (III) in addition to the expected product 1, methyl 4-ethyl naphthalene (II) was described. In order to throw more light on this unusual reduction during dehydrogenation, we studied the dehydrogenation of khusilic acid under various other conditions and the results are incorporated in the present communication.

Khusilic acid (I) upon dehydrogenation with Pd-C (30%) in an atmosphere of carbondioxide at 280° for 20 h leads to the formation of only one naphthalene on the basis of TLC (silica gel G plates impregnated with trinitrobenzene) identified as (II). Even after vigorous conditions of this dehydrogenation not even a trace of (III) was formed. This observation is in complete accord with the literature[2], since the reduction of a carboxyl group to a methyl has been only observed during dehydrogenation using selenium.

When the dehydrogenation of khusilic acid was carried out with equal quantity of Pd-C 30% at 280° for 15 min, it afforded a crystalline acid ($C_{14}H_{14}O_2$ m.p. 185°) in quantitative yields. The IR-spectrum of the acid showed intense bands at 3050 (bonded OH of a carboxyl group), 1680 (Ar-COOH) and at 1615, 1585, 1510, 832 and 752 cm^{-1} (aromatic ring).

The NMR-spectrum of the acid displayed a triplet at 1.42 δ (J = 7 Hz, $-CH_2-CH_3$) a quartet at 3.2 δ (J = 7 Hz, $-CH_2-CH_3$) a singlet at 2.71 δ (Ar $-CH_3$) a singlet at 7.3 δ (C_2 and C_3 protons) a broadened singlet at 8.1 δ (C_7 and C_8 protons) a broadened singlet due to meta coupling at 8.7 δ (C_5 H) and a broad signal at 10.93 δ ($-COOH$). This spectroscopic data suggested structure (IV) for this compound which has also been confirmed chemically. The acid (IV) on reaction with diazomethane

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