flavone (m.p. 152°C; lit. 151°C[7]). Mild acid hydrolysis (0.1 *N* HCl) gave no intermediate, thus indicating the presence of only one molecule of glucose.

The new glucoside is thus postulated as the 3-monoglucoside of kaempferol-4′,7-dimethyl ether. This is the first report of the 3-glucoside of kaempferol-4′,7-dimethyl ether, which itself is quite rare[5,8,9]. The Rf-values and UV-data of the new glucoside and its aglycone are given in the Table.

Finally, kaempferol-4′,7-dimethyl ether was also isolated in the free form, along with a second aglycone (present in trace amount) which was chromatographically identical with rhamnocitrin. However, complete identity of this trace flavonoid was not confirmed, especially in view of the fact that both isomers rhamnocitrin (kaempferol-7-methyl ether) and kaempferide (kaempferol-4′-methyl ether) are impossible to separate chromatographically[10].

Zusammenfassung. Aus den Blättern von *Tamarix nilotica* wurden die 3 Glukoside von Quercetin, Tamarixetin, Kaempferol und Kaempferol-4′,7-dimethyl-Äther, zusammen mit einigen einfachen phenolischen Verbindungen und Zuckern getrennt und einwandfrei identifiziert.

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Electrophoretic Patterns of Ribonucleases in Normal and Habituated Plant Tissues

The existence of a correlation between RNase activity and tumorous transformation has been suggested by many authors (for a review of the subject see Roth[1]); however, the results so far obtained seem to be rather contradictory. Some authors found increased RNase activity in tumorous tissue, others the opposite[2,3].

Geri et al.[4] showed differences in RNase complements between 2 *Nicotiana* species and their tumorous hybrid as far as pH optima, electrophoretic pattern, *para*chloromercuribenzoate (*p*-CMB) induced activation were concerned.

In this context it seemed useful to us to investigate the differences in RNase complements between normal and habituated plant tissue, both grown in vitro: the phenomenon of habituation is defined as the acquired ability of plant tissue culture to synthetize substances (i.e. hormones) which are usually necessary for the continuous proliferation[5]. Such a study could throw some light on the differences, as far as RNases are concerned, between normal tissue and hormone induced tumor[6,7] having the same genetic background and growing in the same, controlled environmental conditions.

Materials and methods. Nicotiana glauca and *Haplopappus gracilis* normal and habituated tissues and *Nicotiana bigelovii* habituated tissues were grown on Linsmajer and Skoog[8] basic substrate supplemented with 2,4-dichlorophenoxyacetic acid (0.4 ppm) in the case of *Nicotiana glauca* and with kinetin (0.02 ppm) and naphtalenacetic acid (1 ppm) for *Haplopappus gracilis* tissue. Habituated tissues were grown on Linsmajer and Skoog's medium without supplements. Due to the great dedifferentiating ability of this plant[6], it was not possible to obtain normal *Nicotiana bigelovii* tissue in culture. All tissues were kept under controlled temperature (25°C), humidity (40%) and light conditions.

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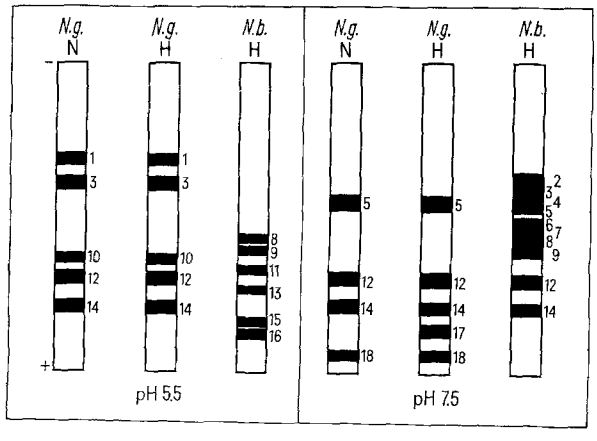

Fig. 1. Electrophoretic patterns on acrylamide gels of RNases extracted from normal (N) and habituated (H) tissues of *Nicotiana glauca* (*N.g.*) and *Nicotiana bigelovii* (*N.b.*), grown in vitro.

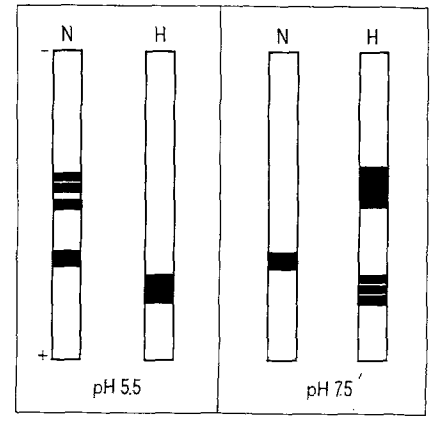

Fig. 2. Electrophoretic patterns of RNases extracted from normal (N) and habituated (H) tissues of *Haplopappus gracilis*. Incubation as in Figure 1.

Tissue samples for enzyme extraction were collected the 12th day after each transfer, washed twice in distilled water and stored for 24 h at 18°C. Extraction was carried out essentially according to WOLFF[9]. The tissue extracts containing RNases were used for electrophoresis on 7% polyacrylamide gels according to DAVIS[10]. Conditions during the run were the same in WOLFF[9]. Gels were stained according to WILSON[11].

Results and discussion. In Figures 1 and 2 the electrophoretic patterns of the 5 stocks at acid (5.5) and alkaline (7.5) pH are reported.

The results obtained with *N. glauca* seem particularly interesting. Tissue extracts from this species showed 5 identical RNase bands in both normal and habituated tissues, when the test was carried out at pH 5.5; on the other hand, habituated tissue showed 5 bands at pH 7.5, 4 of which were found also in test carried on normal callus and 1 seems to be specific to the autonomous strain: it is worth noting that band 17 also occurs in tumorous hybrid *Nicotiana glauca* × *Nicotiana langsdorffii*[4]. *Nicotiana bigelovii* habituated tissue, showed 6 bands at pH 5.5 and 4 at pH 7.5. Control experiments, carried on habituated tissue grown on hormone supplemented media, gave the same results. The situation was more confuse in the case of *Haplopappus gracilis*: habituation in this case seemed to lead to drastic changes involving the whole RNase complement as judged from the electrophoretic behaviour. No single band specific to the transformation to autotrophy could be found.

The data so far reported seem to confirm the differences in RNase complements and activity, between normal and tumorous tissues reported by other authors in plant material[3,4], pointing out the considerable complexity of the problem in some cases as in the *Haplopappus gracilis* situation.

The results obtained with *Nicotiana glauca* tissues, moreover, suggest that isolation and characterization of the specific RNase band observed on habituated material may give some better clue to the problem of RNase involvement in the process of transformation to autotrophy.

Riassunto. É stata fatta un'indagine elettroforetica delle RNasi di tessuti vegetali normali ed abituati di *Haplopappus gracilis*, di *Nicotiana glauca* e di *Nicotiana bigelovii*, coltivati in vitro. Sono state notate delle differenze nei complementi RNasici dei tessuti abituati rispetto a quelli dei tessuti normali. I risultati sono discussi brevemente in relazione alle loro implicazioni col problema dei tumori nelle piante.

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Failure of Carbon Tetrachloride (CCl_4) and Trichlorobromo-methane (CCl_3Br) to Alter Polyribosomal Profiles in the Rat Brain

Studies on CCl_4 and CCl_3Br toxicity are mainly concerned with liver toxicity[1]. The homolytic cleavage of halomethanes to free radicals takes place in the drug metabolizing enzyme system (DMES) in the liver[1]. Drugs inducing or depressing liver DMES increase or decrease CCl_4 toxicity, respectively[2,3], therefore liver toxicity is considered to be secondary to the formation of free radicals. Conversely, toxicity by CCl_4 in a given organ might be considered a sensitive index of the presence of DMES in that organ. RECKNAGEL and GOSHAL[4] found neither in the kidney nor in the brain the changes (proxidant effects) that were observed in the liver of CCl_4-treated animals. Morevor, these authors demonstrated that CCl_4 is not metabolized by isolated rat liver microsomes unless the liver supernatant fraction is added[4].

The present experiments were carried out to study the effect of 2 chlorinated hydrocarbons, CCl_4 and CCl_3Br, on polyribosome profiles of brain homogenates. In fact polyribosomal breakdown is an early effect produced by CCl_4 and CCl_3Br in the liver and is a very sensitive index of the formation of free radical[5].

Materials and methods. Wistar rats of both sexes, weighing 250–300 g, were used. They were fed with a semisynthetic diet (Vogt-Möller, Ditta Piccioni, Brescia, Italy). The animals were starved 12 h before sacrifice, water was given ad libitum. Phenobarbital (PB), as sodium salt, dissolved in 0.9% NaCl, was administered i.p. at the dose of 80 mg/kg of body wt daily for 3 days. The last dose was given 24 h before intoxication; CCl_4 and CCl_3Br were administered at the dose of 0.25 ml/100 g, by stomach tube to untreated and PB-treated animals. All rats were killed by bleeding 2 h after intoxication. Brain polysomal profiles were obtained according to the method of WEISS et al.[6], modified as follows: after killing, the brains were taken and homogenized in 2 volumes of medium S_5 (50 m*M* *Tris*/HCl; 80 m*M* KCl; 5 m*M* $MgCl_2$; 5 m*M* mercaptoethanol and 250 m*M* sucrose). The homogenate was then centrifuged at 13,000 × *g* for 20 min. Aliquots of the postmitochondrial supernatant, treated with sodium deoxycholate to a final concentration of 1%, was layered on to a linear 15%–50% (w/v) sucrose gradient and centrifuged at 2000,00 × *g* for 40 min in a Spinco SW50 rotor at 0°C. The extinction profiles at 260 nm were recorded automatically with a Unicam model SP1800 spectrophotometer, by pumping the gradient trough a flow cell at constant flow rate. Liver polysomal profiles were obtained as follows: after killing, the liver was taken and homogenized in 2 volumes of medium S_5. The homogenate was then centrifuged at 20,000 × *g* for 20 min. Aliquots of the postmitochondrial supernatant, treated with sodium deoxycholate to a final concentration of 1%, were layered on to a linear 15%–50% (w/v) sucrose

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