Vincadifformin und Minovin, zwei weitere racemische Alkaloide aus *Vinca minor* L.[1]

In einer vorangegangenen Mitteilung beschrieben wir die Isolierung des racemischen Alkaloides Vincanorin[2] aus *Vinca minor* L. und dessen Strukturaufklärung[1,3,4]. Aus der schwach basischen Fraktion T[3] der Alkaloide aus *Vinca minor* L. erhielten wir durch Säulenchromatographie an Aluminiumoxyd (Benzol-Petroläther 1:1) ausser Vincaminorein[5,6] auch das racemische Vincadifformin, welches vor kurzem von JANOT et al.[7] aus *Vinca difformis* Pourr. isoliert[8] und in seiner Struktur erkannt wurde[9].

Beim Vergleich der Werte des Vincadifformins mit einem anderen α-Methylenindolinalkaloid Minovin[10] zeigte sich, dass letzteres das ind-N-Methylderivat des ersteren ist. Hierauf wiesen die Bruttoformel, die Verschiebung des Ultraviolettspektrums und die Abwesenheit der NH-Bande im Infrarotspektrum sowie der Nullwert der spezifischen Drehung. Durch Methylierung von Vincadifformin mit Methyljodid in flüssigem Ammoniak erhielten wir eine Verbindung, Smp. 120–122° (sintert ab 98°), $[\alpha]_D = 0°$ (Chloroform, Äthanol), für $C_{22}H_{28}N_2O_2$ (352,5) ber. C 74,96; H 8,01; N 7,95%; gef. C 74,81; H 7,93; N 8,07%, deren UV- und IR-Spektren mit denen des Minovins identisch sind. Die Identität beider Stoffe ergab sich durch direkten Vergleich. Vincadifformin und Minovin stehen zueinander im selben Verhältnis wie Vincadin und Vincaminorein[6]. Einen zusätzlichen Beweis liefert das Massenspektrogramm[11] des Minovins; es zeigt ein Molekulargewicht von 352, also 14 mehr als Vincadifformin (Mol.-gew. 338[9]), während sich der einzige sehr intensive Bruchstück-peak wie im Spektrum von Vincadifformin bei m/e 124 befindet. Das zusätzliche Kohlenstoffatom des Minovins muss sich daher im Indolinsystem befinden, was durch den Peak bei 3,30 δ (Singlett, drei Wasserstoffatome) im NMR-Spektrum belegt wird.

Racemische Alkaloide wurden in der grossen Gruppe der Indolalkaloide verhältnismässig selten[9,12,13] gefunden. Dass in *Vinca minor* L. gleich drei racemische Alkaloide, deren pentacyclische Skelette die Möglichkeit einer Racemisation ausschliessen, gefunden wurden, legt die Vermutung nahe, dass die Entstehung im Schema ihrer Biogenese zu suchen ist. Wir glauben, dass diese Pflanzengattung für Versuche in dieser Richtung geeignet ist.

Summary. From *Vinca minor* L. two additional racemic alkaloids of the α-methylenindoline type were obtained: vincadifformine (previously known from *Vinca difformis* Pourr.) and minovine which is shown to be its ind-N-methyl derivative.

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Influence of Nutritional Conditions on the Cellular RNA Metabolism *in vivo* and *in vitro*

Three types of ribonucleic acid have been recognized in living cells: soluble, ribosomal and messenger RNA[1]. Soluble and ribosomal RNA can be separated by column chromatography on methyl-esterified serum albumin[2,3]. On applying this method, a further RNA fraction could be identified which probably plays the role of messenger RNA in virus-infected and uninfected human amnion cells[4]. Further studies concerning the influence of unfavourable growth conditions on RNA metabolism in mammalian cells provided evidence for the synthesis of another highly polymerized RNA. Some representative results are reported here.

Two separate primary cultures of embryonal mouse fibroblasts were incubated in a pH-stat at 36°C in Eagle's medium supplemented with ^{32}P-orthophosphate: the first, containing 5×10^6 cells/ml ('concentrated' cell suspension), was incubated for 16 h with 17 μc ^{32}P/ml; the second, containing 1.7×10^5 cells/ml ('diluted' cell suspension), was incubated for 6.5 h with 2.5 μc ^{32}P/ml (after further incubation of the 'diluted' cell suspension messenger RNA proportionally no longer contained more ^{32}P than ribosomal RNA and was, therefore, no longer recognizable on the elution diagram).

As another system, tumor cells from Ehrlich ascites carcinoma bearing mice were taken out on the 3rd ('early') and on the 11th ('late') day after passage. 6 h before collecting the cells 200 μc of ^{32}P were injected subcutaneously nto each mouse. RNA from these four cell suspensions was isolated and subsequently chromatographed as previously described[5], except that the column was washed after loading with 100 ml of solution of the initial salt concentration in order to remove inorganic ^{32}P, nucleotides and highly labelled low polymerized nucleic acids (e.g. soluble RNA). UV-absorbancy and ^{32}P content of the eluting RNA were determined as described[5].

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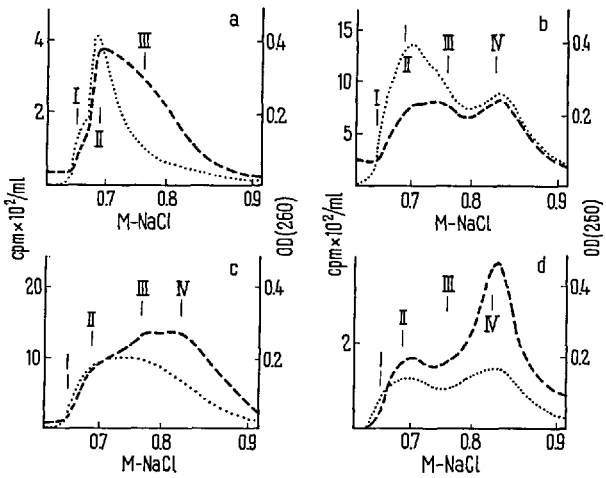
The RNA elution diagrams are presented in Figure a–d. The previously characterized RNA fractions are labelled as I (16s), II (23s)[5] and III (unstable, presumably messenger RNA[4]). An additional highly ^{32}P labelled RNA fraction IV, which elutes later than all other RNAs, is clearly recognizable in Figure b ('concentrated' cell culture) and d ('late' tumor cells), present in small amounts in c ('early' tumor cells) and absent in a ('diluted' cell culture). Since the applied chromatographic method separates the nucleic acids according to their chain length, the RNA fraction IV is considered to be of higher molecular weight. The region of its appearance in the elution diagram is comparable with the one of polio virus RNA[6].



Elution diagrams of RNA obtained from: a, 'diluted' cell suspension; b, 'concentrated' cell suspension; c, Ehrlich ascites tumor cells 'early'; d, Ehrlich ascites tumor cells 'late'. 0.8, 2.7, 2.7 and 2 mg of RNA were loaded onto the column in the experiments 'a', 'b', 'c' and 'd' respectively. For further details see text. ···· ultraviolet absorbancy at 260 mμ. ---- radioactivity, cpm-counts per min (please note that the radioactivity scale differs with every picture). The Roman numbers correspond to the following fractions on the diagram: I: 16 s, ribosomal RNA; II: 23 s, ribosomal RNA; III: unstable, presumably messenger RNA; IV: highly polymerized RNA described in the text (for additional information on fractions I–III see[3–5]).

The RNA from fraction IV is also more stable than the one from fraction III. The latter is completely broken whereas the former remains intact to 70% when incubated at 23°C for 60 min[4].

The synthesis of this RNA eluting last was observed also in other animal cells under unfavourable growth conditions, e.g. in human amnion cells during incubation in buffered saline, incomplete medium and in highly concentrated cell suspension. In tumor-bearing mice, this highly polymerized RNA was not only recognizable in neoplastic cells but also increased steadily in the liver until death.

On the other hand, no synthesis of this RNA was detectable by our methods in cells under optimal growth conditions. Investigations are under way to obtain information on the biological role of this RNA[7].

Zusammenfassung. Es wird der Einfluss von Stoffwechselbedingungen auf die RNS-Synthese in intakten Zellen *in vivo* und *in vitro* mittels ^{32}P-Markierung und einer säulenchromatographischen Fraktionierung der isolierten RNS untersucht.

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Accurate Determination of Total CO_2 in the Brain

All previous measurements of tissue carbon dioxide contents have been performed on excised tissues, in which *post mortem* changes might seriously have influenced the true *in vivo* levels of the labile carbon dioxide compounds. In order to avoid such changes, a method was developed which allows the determination of the total carbon dioxide content of tissues, frozen *in situ*. The results with this method indicate that the majority of the values reported earlier are too high and, further, that part of the difference is due to *post mortem* changes.

Methods. The experiments were performed on rats of the Sprague–Dawley strain which were anaesthetized with Nembutal (40–50 mg/kg body weight), tracheotomized and allowed to breathe spontaneously. The carbon dioxide tension of the blood was measured on samples drawn from a cannula in the femoral artery using the micromethod of SIGGAARD ANDERSEN et al.[1]. Immediately after the analysis, the brain was frozen *in situ* by plunging the whole animal into liquid nitrogen. When *post mortem* changes were to be investigated, the blood sampling preceded decapitation of the animal and rapid excision of the tissue, which was then placed in the liquid nitrogen. In some experiments, the period between the decapitation and the freezing of the tissue was deliberately prolonged so as to give delays of up to 10 min. In the group of animals analysed after quick-freezing, the brains were separated from the carcasses in the frozen state, using chisel and hammer. In all cases the supratentorial parts of the brain were used for the analyses.

The subsequent handling of the brain samples occurred in a Perspex box, manipulations of the samples being carried out with neoprene gloves. The box was continuously perfused with nitrogen gas which had previously been passed through devices for absorbing carbon dioxide and water vapour. The brain samples were transferred to a stainless steel mortar and, while still in liquid nitrogen, ground to a fine powder. Aliquots of the powder were added to special diffusion chambers mounted on Pyrex lids which had been blown and ground to fit commercial Conway 2-A units (Gallenkamp, London)[2]. The chambers contained a 5% trichloracetic acid solution, which could be stirred continuously. The amount of tissue added to the chambers was weighed by difference to the nearest tenth of a mg. 2 h were allowed for diffusion, the evolved carbon dioxide being captured in $0.2N$ $Ba(OH)_2$, which

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