Cactaceae Alkaloids VIII. N-Methyl-4-Methoxyphenethylamine from *Lepidocoryphantha runyonii* (Br. and R.) Backbg.

Lepidocoryphantha runyonii (Br. and R.) Backbg. (synonym: *Coryphantha runyonii* Br. and R.) and the only other species of this genus, *L. macromeris* (Eng.). Backbg. (T.) are the only cacti known to contain the alkaloid macromerine[1,2].

In a recent investigation[3], with the object to discover potential precursors of macromerine, we showed the presence in *L. runyonii* also of tyramine, hordenine and N-methyl-3,4-dimethoxyphenethylamine together with some minor unknown components. We have now identified one of these unknown compounds as N-methyl-4-methoxyphenethylamine, a compound previously not known to occur in nature. Its identity was established as follows.

The alkaloids were extracted from fresh *L. runyonii* and purified[3], care being taken not to loose any volatile amines. The alkaloid fraction was analyzed by GLC using SE-30 and XE-60 columns[3] and also by gas chromatography – mass spectrometry (LKB 9000 instrument)[3]. Gas chromatogram of alkaloid fraction analyzed from *L. runyonii*: 5% SE-30 on Gas Chrom P; 120 °C isothermal for 10 min followed by temperature programming, 4 °/min. The mass spectrum of the compound showed a mol. wt. (M^+) of 165 (1%), a base peak at m/e 44 (100%), and major peaks at m/e 121 (3%) and 122 (31%). This suggested an N-methylated methoxyphenethylamine such as e.g. N-methyl-4-methoxyphenethylamine which was accordingly synthetized[4] and found to be identical (mass spectrum, GLC) with the compound present in *L. runyonii*.

This is apparently the first encounter in nature of a simple monomethoxyphenethylamine. However, monomethoxysubstituted phenylpropane derivatives are known, e.g. anethole and *O*-methyltyrosine, the latter a constituent of the antibiotic puromycin[5]. Recently, we have shown the occurrence[3] in cacti of 3,4-dimethoxyphenethylamine (in *Lophophora williamsii* and *Trichocereus* sp.[3]) and its N-monomethyl (in *Echinocereus merkeri*[6] and *L. runyonii*[3]) and N,N-dimethyl derivatives (in *E. merkeri*[6]).

Zusammenfassung. N-Methyl-4-methoxyphenäthylamin wird zum ersten Mal in der Natur vorkommend, in der Kaktee *Lepidocoryphantha runyonii* nachgewiesen.

ST. AGURELL[7]

University of Uppsala, Faculty of Pharmacy, 113 86 Stockholm (Sweden), 19 June 1969

1 J. E. HODGKINS, S. D. BROWN and J. L. MASSINGILL, Tetrahedron Letters (*1967*), 1321.
2 L. E. BELOW, A. Y. LEUNG, J. L. MCLAUGHLIN and A. G. PAUL, J. Pharm. Sci. *57*, 516 (1968).
3 S. AGURELL, Lloydia *32*, in press (1969).
4 S. KIRKWOOD and L. MARION, J. Am. chem. Soc. *72*, 2522 (1950).
5 M. M. RAO, P. F. REBELLO and B. M. POGELL, J. biol. Chem. *244*, 112 (1969).
6 S. AGURELL, J. LUNDSTRÖM and A. MASOUD, in press J. Am. pharm Ase.
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Association of Viral Ribonucleoprotein with Polysomes

The interaction of cellular ribonucleoprotein with ribosomes has recently been described[1–3]. The present paper reports the formation of polysomal complexes with parental viral ribonucleoprotein (RNP) in Sendai virus infected Ehrlich ascites tumor cells. The synthesis of virus-specific RNA and viral structural proteins with the formation of infectious RNP was described in this system[4].

Materials and methods. Ehrlich tumor cells pretreated with actinomycin D (2 μg/ml) for 1 h were infected ^{32}P or (^3H) leucine labelled Sendai virus at the multiplicity of about 100 EID_{50} per cell. 1 h after infection the cells were exposed to (^3H) leucine (2 μC/ml) for 10 min, then quickly chilled, washed with RSB, resuspended in 2 ml of the same and disrupted in Dounce homogenizer. 2 ml of the supernatant was layered on to 17–40% sucrose density gradient prepared in the same buffer and was centrifuged at 25,000 rpm for 3 h at + 2 °C. Preformed CsCl density gradients[6,7] (5 ml) were centrifuged at 36,000 rpm for 15 h. Radioactivity in fractions precipitated with trichloracetic acid was measured in a Packard liquid scintillation spectrometer.

Results and discussion. Figure 1a shows the sedimentation pattern of ^{32}P and ^3H input viral radioactivity 1 h after cell infection with ^{32}P and (^3H) leucine labelled virus. It can be seen that both viral labels are coincident and recovered in the polysome region in nearly the same position as RNP of Sendai virus obtained by the treatment of ^{32}P-labelled virus with 0.5% sodium deoxycholate (Figure 1b), but the profiles of input viral radioactivity in homogenates are somewhat more heterogeneous as compared with viral RNP. Thus, Sendai virus RNP appears to enter the cells and to be slightly changed for the first hour of incubation.

To determine whether the viral label is associated with polysomes, homogenates from the cells infected with ^{32}P-virus and exposed in 1 h to ^3H-leucine (2 μC/ml) were centrifuged, the fractions containing polysomes (from 4 to 9) were pooled, divided into 2 equal parts, 1 of them being treated before resedimentation with EDTA (0.01 *M*) and the other used as a control. Figures 1c and

1 W. A. CARTER and N. B. LEVY, Arch. Biochem. Biophys. *120*, 562 (1967).
2 E. C. HENSHAW, J. molec. Biol. *36*, 401 (1968).
3 R. P. PERRY and D. E. KELLEY, J. molec. Biol. *35*, 37 (1968).
4 A. G. BUKRINSKAYA, S. M. KLIMENKO, JU. A. SMIRNOV and B. V. GUSCHIN, J. Virol. *2*, 752 (1968).
5 R. D. BARRY and A. G. BUKRINSKAYA, J. gen. Virol. *2*, 71 (1968).
6 A. S. SPIRIN, N. V. BELITSINA and M. J. LERMAN, J. molec. Biol. *14*, 611 (1965).
7 J. B. IFFT, D. H. VOCT and J. VINOGRAD, J. phys. Chem. *65*, 1138 (1961).

d demonstrate that EDTA eliminates the incorporated amino acid label from the polysome region, and input viral radioactivity is released as polydisperse array of RNP particles.

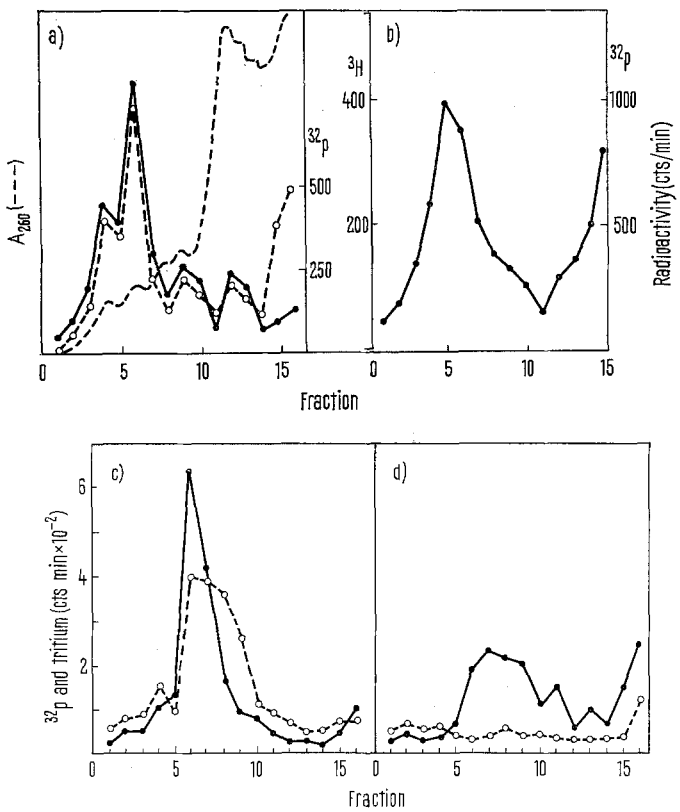

Fig. 1. Distribution of ^{32}P (-●—●-) and tritium (-○—○-) after sucrose gradient centrifugation of: (a) cytoplasmic extracts obtained 1 h after cell infection with virus labelled with ^{32}P and [^3H] leucine; (b) ^{32}P labelled virus treated with 0.5% sodium deoxycholate; (c) fractions of sucrose gradient containing polysomes (4–9) from the gradient after centrifugation of cytoplasmic extracts of cells infected with ^{32}P-labelled virus and pulse-labelled for 10 min with [^3H] leucine; (d) the same material treated before resedimentation with 0.01 M EDTA.

The association of viral RNP with polysomes was confirmed by buoyant density studies in CsCl density gradient. As seen in Figure 2a, polysomes from non-infected cells give a single band in CsCl at $\delta = 1.52$. When RNP isolated from ^{32}P-labelled virus and purified by sedimentation through 0.5–2.0 M sucrose has been added to such polysomes prior to fixation with formaldehyde, tritium label shows 2 density peaks at $\delta = 1.52$ and 1.49 (Figure 2b). Viral label gives a principal density band at $\delta = 1.35$ (viral RNP gives a band at $\delta = 1.31$) and 2 smaller bands at densities of $\delta = 1.52$ and 1.49, coincided with tritium peaks. These 2 peaks completely disappear when the material is treated with EDTA (0.01 M) prior to fixation with formaldehyde (Figure 2c).

Tritium label from infected cell homogenates exhibits 4 peaks at $\delta = 1.53$–1.55, 1.49, 1.41 and 1.37 g/cm^3. Viral label shows a principal density band at $\varrho = 1.37$ and 2 comparatively smaller bands at densities of $\delta = 1.55$ and 1.49 coincided with protein label. An additional peak of ^3H and ^{32}P at $\delta = 1.45$ g/cm^3 was also observed in a number of experiments.

The data presented suggest that partly deproteinized viral RNP with $\delta = 1.35$–1.37 instead of 1.31 g/cm^3 is associated with ribosomes forming complexes of 2 species: at the same density as cellular polysomes and monosomes ($\delta = 1.52$–1.55 g/cm^3) and at lower density ($\delta = 1.49$ and 1.45 g/cm^3). RNP structures strongly resembling Sendai virus RNP were discovered in the gradient fractions containing these species when examined under electron microscope.

Выводы. при заражении клеток асцитной карциномы Эрлиха вирусом Сендай вирусный рибонуклеопротеид (РНП) обнаруживался в цитоплазме по крайней мере в течение 6 часов после заражения. Частично депротеинизированный РНП обладал способностью ассоциироваться с рибосомами, образуя комплексы с разной плавучей плотностью (δ=1,41, 1,45, 1,49 и 1,55 g/cm^3). При добавлении РНП к экстрактам нормальных клеток также наблюдалось образование комплексов его с рибосомами.

A. G. Bukrinskaya and V. M. Zhdanov

D. I. Ivanovsky Institute of Virology, USSR Academy of Medical Sciences, Moscow (USSR), 21 April 1969

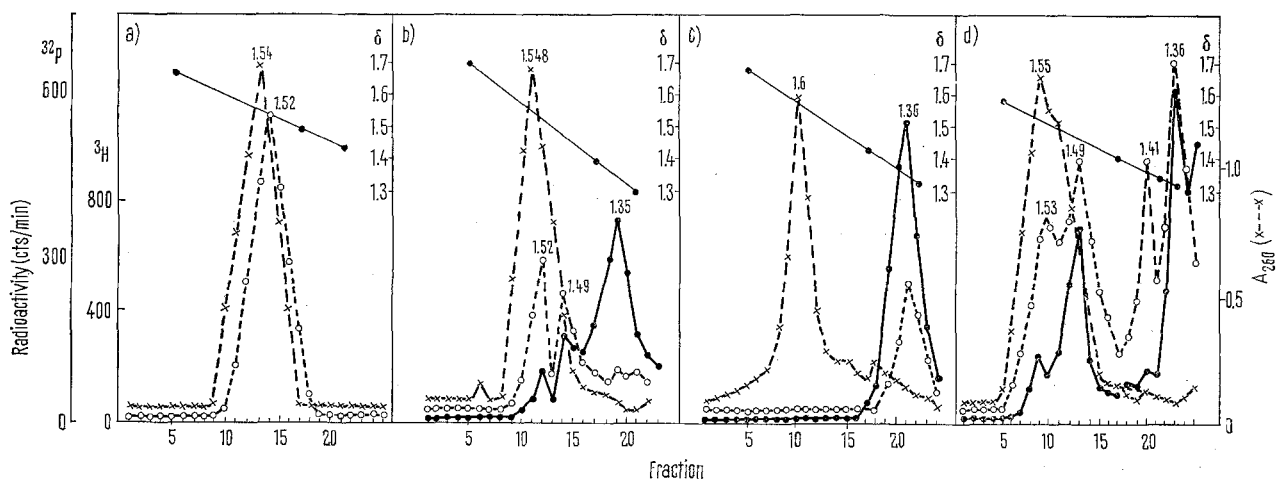

Fig. 2. Buoyant densities of polysomes extracted from cells pulse-labelled for 10 min with [^3H] leucine: (a) polysomes from non-infected cells; (b) polysomes from non-infected cells mixed prior to fixation with ^{32}P-labelled viral RNP; (c) the same polysomes mixed with viral RNP and treated with 01 M EDTA; (d) polysomes extracted from cells 1 h after infection with ^{32}P-labelled virus. -●—●-, ^{32}P; -○—○-, tritium.