all parameter changes results in a new locus of stability.

The elevation of pyruvic carboxylase in alloxan diabetes may represent a mechanism whereby the concentration of intermediates of the tricarboxylic acid cycle is maintained despite the production by the liver of large amounts of glucose. It is noteworthy that the effect of the diabetic state on enzyme activity is reversed by the administration of protamine zinc insulin.

Note added in proof. After this manuscript was submitted for publication, a report was published by Wagle (*9*) on the effect of diabetes on pyruvic carboxylase activity which is in agreement with the data on diabetes presented here.

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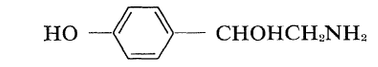
6 January 1964

l-Octopamine in Citrus: Isolation and Identification

Abstract. l-*Octopamine*, [l-p-*hydroxy-α-(aminomethyl)benzyl alcohol*], *has been isolated and identified from extracts of juice and leaves of the Meyer lemon. Identification was by chromatography, optical rotation, ultraviolet absorption curves, fluorescence spectra, and infrared spectroscopy.* l-*Octopamine has not previously been isolated and identified from plants.*

While making studies on the identification of *l*-synephrine in citrus (*1, 2*), a brown ninhydrin spot was observed on paper chromatograms of extracts of the juice and leaves of Meyer lemon (*Citrus spp.*) (*3*). After extraction and purification, this spot was identified by infrared analysis and optical rotation as *l*-octopamine, [*l-p*-hydroxy-α-(aminomethyl)benzyl alcohol], also called norsynephrine and *p*-hydroxymandelamine.



l-Octopamine was extracted from Meyer lemon leaves that had been frozen shortly after picking. The extraction procedure was similar to that used for *l*-synephrine (*1*), except that the solvent consisted of a mixture of 80 percent methanol and 20 percent water. After eluting the basic compounds from the ion-exchange column, the mixture was evaporated to a small volume under reduced pressure at 60°C. In some instances, a few milligrams of sodium metabisulfite was added to the extract as an antioxidant before extraction and after passage through the column. The eluate was diluted with methanol and treated directly with oxalic acid as previously described (*1*). A mixture of crystalline oxalates of several nitrogen compounds was thus obtained. Tyramine was identified by infrared analysis and *l*-synephrine by its paper chromatographic R_F values and by its characteristic reddish brown ninhydrin color.

l-Octopamine was separated from the other constituents by chromatography on a column (10 by 80 mm) of ion exchange resin (Dowex 50W-X8, 200–400 mesh in the ammonium form). The resin was prepared as previously described (*1*) except that it was washed until the eluate was neutral. The oxalate mixture was dissolved in water, placed on the column, and eluted with a gradient produced by slowly adding 3*N* NH_4OH to 1 liter of water. Fractions (10 ml) were collected and *l*-octopamine was eluted approximately after 300 ml of eluting solution was added as indicated by ultraviolet absorption at 290 mμ and paper chromatography. Fractions containing *l*-octopamine were taken to dryness and the residue was sublimed in a vacuum at 0.1 mm-Hg and 120°C. Authentic *dl*- or *l*-octopamine purified in this manner was not altered.

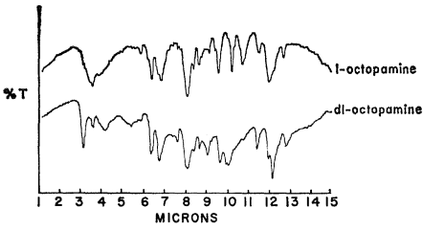


Fig. 1. Infrared absorption curves of *l*-octopamine and *dl*-octopamine (*%T*, percent transmission).

Several methods were used in identifying *l*-octopamine, including the aforementioned chromatographic column system in which the unknown separated into the same fractions as an authentic sample of *dl*-octopamine. Separation was made with paper chromatography with three solvent systems. *l*- and *dl*-Octopamine had an R_F of 0.37 with an EDTA-buffered paper chromatographic method (*4*). When a known sample of octopamine was cochromatographed with the unknown from citrus leaves, the R_F values and color were the same. Winitz (*5*) and others have pointed out that it is not rare for nitrogen compounds of varying structure to possess identical mobilities in a number of different solvent systems. For this reason, methods other than chromatography were also used for identification.

Ultraviolet absorption curves of *dl*-octopamine and the isolated compound were identical. In 0.1*N* HCl, absorption peaks were at 222 and 273 mμ; in phosphate buffer *p*H 12, peaks were at 241 and 290 mμ. Fluorescence spectra of *dl*-octopamine and the isolated compound in 0.1*N* HCl were also identical with the excitation peak at 270 mμ and the emission peak at 310 mμ. However, similarities in absorption and fluorescence characteristics of *l*-octopamine and *l*-synephrine precluded the use of such characteristics in a positive identification of *l*-octopamine. Infrared absorption curves were made of the extracted free base and compared with an authentic sample of *dl*-octopamine. The general shape of the curves was similar but there were distinct differences in the regions of 3, 6.5–7.2, and 9.5–10.5 μ (Fig. 1). Similar differences between the infrared curves of *l*-synephrine and the racemic form were reported previously (*1*). Finally, a synthetic sample of *l*-octopamine was compared with the naturally occurring compound and the infrared absorption curves were identical in all regions. *l*-Octopamine and the compound isolated from citrus decomposed at 167° to 168°C when heated at the rate of approximately 3°C per minute.

The octopamine extracted from citrus leaves was the levoisomer with an optical rotation in 0.5*N* HCl $[\alpha]_D^{25}$ of −41. The optical rotation of naturally

occurring octopamine has not been previously determined. Esparmer's "assumption" (*6*) that the levo form was present in the octopus was based on blood pressure studies. However, his proof did not distinguish between the levo and dextro isomers. The isomer in animals is yet to be determined. Octopamine has not been previously isolated and identified from plants. Gjessing and Armstrong (*7*) reported finding in oranges "small amounts of a substance which has chromatographic properties of octopamine." However, these workers offered no further explanation.

This amine was first extracted from the salivary glands of octopus, from which the name was derived (*6*). Later it was reported in human and animal urine (*8*). Pisano *et al.* (*9*) have pointed out that the significance of synephrine and octopamine in animals is not known, but that these compounds are fairly active pharmacologic agents. Furthermore, they are readily transformed into adrenaline and noradrenaline, respectively, by enzymatic action or ultraviolet radiation (*6, 10*). The presence of these physiologically active amines in plants will make it more difficult to determine if these compounds and their metabolites in animals are from dietary or metabolic sources.

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Pacemaker Neurons: Effects of Regularly Spaced Synaptic Input

Abstract. *The consequences of inhibitory or excitatory synaptic input between pacemaker neurons were predicted mathematically and through digital-computer simulations, and the predicted behavior was found to occur in abdominal ganglia of* Aplysia *and in stretch receptors of* Procambarus. *Discharge patterns under conditions that do not involve interneuronal feedback are characteristic and self-stabilizing. Paradoxically, increased arrival rates of inhibitory input can increase firing rates, and increased excitatory input rates can decrease firing rates.*

A neuron is known as a pacemaker when each impulse it generates is followed first by a repolarization and then by a depolarizing drift of the membrane potential, which again reaches threshold, thereby spontaneously producing a regular sequence of impulses. Such a neuron may also receive input (Fig. 1) in the form of inhibitory (IPSP) or excitatory (EPSP) postsynaptic potentials (*1*). Since an EPSP generally reduces the time required to reach threshold level and an IPSP prolongs this time, intervals containing EPSP's are shorter than the "natural" intervals (those with no postsynaptic potentials), and those containing IPSP's are longer (Fig. 1*a*). Mathematical descriptions, extended by digital-computer simulations, predict distinct and patterned sequences of impulses in a pacemaker cell subjected to certain regularly spaced IPSP's or EPSP's of constant magnitude, such as would be generated by other pacemaker cells. These predictions were confirmed by experiments in the sea slug (*Aplysia californica*) and crayfish (*Procambarus clarkii*).

The basic condition for the production of such responses is the functional dependence of the delay (that is, the amount by which the natural interspike interval is modified by a postsynaptic potential) upon the latency of the postsynaptic potential (that is, the time elapsed between the previous spike and the arrival of the next postsynaptic potential). Experimental delay-function curves were similar for the two species; Fig. 2 shows typical examples for an IPSP. The interspike interval as modified by a single postsynaptic potential is given by

$$s = \sigma + f(\phi), \qquad (1)$$

where σ is the "natural" interspike interval of the pacemaker, ϕ the latency, and $f(\phi)$ the delay caused by a particular postsynaptic potential. If the interval τ between successive postsynaptic potentials from the same source is constant and sufficiently long, so that at most one postsynaptic potential arrives between successive pacemaker firings, then an arrival at latency ϕ_1 will be followed, after the next spike, by an arrival at a latency

$$\phi_{i+1} = \phi_i + \tau - \sigma - f(\phi_i). \qquad (2)$$

The sequence of latencies $\phi_1, \phi_2, \ldots$, converges to a limiting stable value ϕ_∞ if the derivative of the delay function $df(\phi)/d\phi$ is positive and less than 2 (*2*). The approach to ϕ_∞ is monotonic if the derivative is less than 1, oscillatory if greater than 1.

The interval τ is related to the stable latency by

$$\tau = \sigma + f(\phi_\infty), \qquad (3)$$

and hence the stable latency is given by

$$\phi_\infty = f^{-1}(\tau - \sigma). \qquad (4)$$

The domain of stability for τ (or for frequency $\nu = 1/\tau$) is that set of values for which the delay function $f(\tau - \sigma)$ has an inverse, and has a slope between 0 and 2. Similar criteria can be derived for intervals in which more than one postsynaptic potential arrives between successive firings. The delay function satisfies these criteria for a single arriving IPSP when the latency lies between ρ (the length of the absolute refractory period, before which time an IPSP does not delay firing) and σ, the natural interspike interval, at which time firing will occur spontaneously. The domain of stability is then given by

$$\sigma + f(\rho) \leqq \tau < \sigma + f(\sigma). \qquad (5)$$

At all intervals between arrivals within this zone, the IPSP latency will become stable, and the cell's spikes will "lock in" with the IPSP's in a one-to-one manner, not in synchrony but delayed by the stabilized latency, as shown in Fig. 1*c*. If the IPSP frequency is increased, the activity enters an "un-