sively, in average 0.9 Hz/h. The lowest frequency we could find was 11 Hz on 1 animal after 21 h registration. The amplitude (unipolar lead) was found not to exceed 100 μV, usually being between 30 and 50 μV.

The cortical EEG of these animals revealed a rhythmic activity (Figure 1) from the beginning of registration. Not only was the frequency of this ryhthm identical with the above-mentioned cerebellar one, but the coherency was also highly significant (Figure 2). The cortical rhythm was most obvious at the precentral area because of its high amplitude (up to 120 μV) and the less striking theta rhythm in this lead.

In 3 experiments, mid-brain transsection was performed. In all 3 cases, a pattern of EEG at rest resulted instead of the previous cortical rhythmic activity. In one of these experiments, we were able to record regular cerebellar activity following the transsection.

A preliminary evaluation of cortical phase relationship revealed a phase shift only in the anterior-posterior direction with the rhythm being in phase at electrodes placed symmetrically at both hemispheres. The phase relation between cerebellar rhythm and any reference point (in Figure 2 the precentral lead) depends on the depth of the cerebellar electrode, thus indicating phase differences within the cerebellum.

Transition stage. On 3 animals we were able to study the development of cortical and cerebellar rhythmic activity. In a typical experiment, a rabbit was injected with 10^4 LD_{50} tetanus toxin i.v. Surgical procedures were started 18 h later, in the presence of symptoms of reluctance to move and stiffness of gait. At the beginning of registration, only an inconspicuous cerebellar rhythm of 35 Hz was present. It became more regular as the experiment proceeded. The cortical EEG was characterized at the beginning by slow waves and precentral spindles. In the course of a few h, the picture changed to cortical desynchronization, concomitant with continuous hippocampal theta rhythm, and 10 h later the above described rhythm was established.

Local tetanus. Cortical or cerebellar rhythmic activity could not be seen in either brainstem tetanus or in local tetanus of the limbs.

Discussion. Cerebellar rhythmic activity is not specific for tetanus intoxication. It occurs under the influence of 'barbiturate like agents'[5] and strychnine[5–8] and may be induced by arousal stimulation[7,8]. From the latter condition, it can be clearly distinguished by its regularity and the occurence of lower frequencies. As noticed at the transition stage, we never saw a sudden change from less regular to the highly rhythmic cerebellar activity. We may therefore assume that the cerebellar rhythm of tetanus intoxication developes gradually. Our experimental setup does not permit any conclusion as to cause and origin of the cerebellar rhythm. Tetanus toxin may be similar in this respect to strychnine, which is said to induce it by influence on spinal cord and brainstem[6–8], although we were unable to find the cerebellar rhythm in animals with brainstem tetanus.

On the other hand, the rhythmic activity of cortical areas seems to be significant. The toxin, as clearly demonstrated by our mid-brain transsections, does not act directly on cortical structures[9].

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Potentiation of Ethanol Narcosis by Dopamine- and L-DOPA-Based Isoquinolines

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Summary. The isoquinolines, salsolinol and 3-carboxysalsolinol, prolong ethanol-induced narcosis in mice. Pretreatment with carbidopa increases the effect of 3-carboxysalsolinol but not of salsolinol. These results suggest that ethanol sleeping-time potentiation by L-DOPA may involve a partial conversion to the isoquinoline in vivo. A central depressant action of salsolinol or the 3-carboxy analogue is suggested.

Many studies have indicated that neuroamines, or their metabolites, may participate in the narcotic action of ethanol. Several sleeping time studies, in which administrations of ethanol were preceded by injections of dopamine and 5-hydroxytryptamine[2,3] and their precursors, L-DOPA and 5-hydroxytryptophan[4], have shown that these substances significantly prolong the depressant action of the alcohol. Attention has focused, however, on the aberrant reductive pathway metabolites, the arylethanol products, dihydroxyphenylethanol (DOPET) from dopamine and tryptophol and 5-hydroxytryptophol from 5-hydroxytryptamine[3,5]. These metabolites are also able to produce prolongation of ethanol-induced narcosis; yet the results indicate that the neuroamines are more potent potentiators than either the precursors or the reduced metabolites.

Other unusual metabolites may occur under the experimental conditions employed. Acetaldehyde, the prime

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metabolite of ethanol, is known to condense readily both in vitro and in vivo[6-8] with many phenylethylamines, such as the neural catecholamines, to yield simple isoquinolines related to the alkaloid salsoline[9]. The ease of formation of the cyclized condensates suggested that derived isoquinolines may participate in the observed potentiation of the soporific action of ethanol in mice when dopamine or L-DOPA are co-administered[3,4]. To pursue this possibility ethanol narcosis studies were conducted in combination with salsolinol, the condensate from dopamine and acetaldehyde, and 3-carboxysalsolinol, the condensate L-DOPA and acetaldehyde. The structural relations of these compounds are represented in Figure 1.

Male, Swiss-Webster albino mice, ranging in weight from 24 to 32 g were used and the studies were conducted at room temperature[10]. The sleeping time protocol developed by KAKIHANA[11] was employed. Following the drug administrations, which were always given during the late morning, the time at which each mouse lost the righting reflex was recorded. The endpoint of the duration of sleep was considered to occur when the subject had righted himself twice within 30 sec. This method for the determination of the endpoint was chosen to insure that intermittent leg jerks made during sleep did not result in a righting which was actually an artifact. An arbitrary value of 150 min was assigned if the mouse was still asleep at the end of that period of time.

Both salsolinol and 3-carboxysalsolinol as well as the ethanol were prepared in saline. The isoquinolines were administered immediately before the time of the ethanol administrations. The ethanol used in these studies was delivered as a 25% (v/v) solution, a dose of 4 g/kg (87 mM/kg) being given[3,4,10]. Unless otherwise stated, the mode of administration for all drugs was by the i.p. route. For the duration of sleeping time the mean and standard error of the mean were determined, and the level of significance was determined by Student's t-test. The isoquinolines used in these studies were prepared in our laboratory. Salsolinol was made by a method based on the procedure of BUCK[12,13], whereas the carboxylated analogue was synthesised by the method of BROSSI et al.[14].

The various drug treatments involved the injection of either salsolinol or 3-carboxysalsolinol, alone, or in combination with ethanol. In order to obtain a reference sleeping time, ethanol was administered with saline (5 μl/g body weight). The isoquinoline solutions were also delivered in this volume in the conjoint treatments. In subsequent experiments, ethanol narcosis tests were

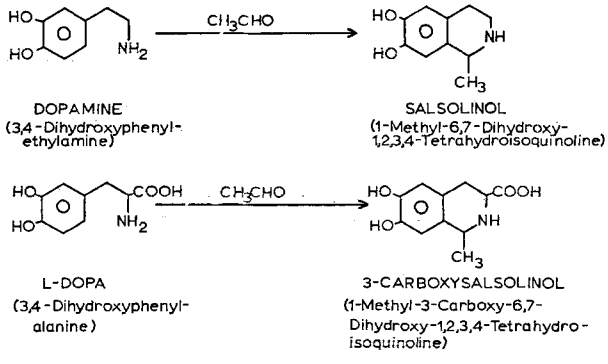

Fig. 1. Structural representations and relations of dopamine and L-DOPA and their tetrahydroisoquinoline derivatives.

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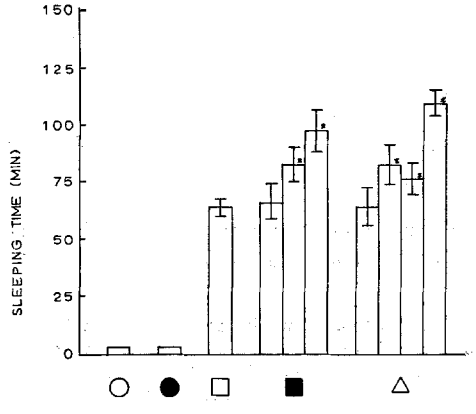

Fig. 2. Potentiation of ethanol-induced narcosis in mice by salsolinol and 3-carboxysalsolinol. Standard errors of means are represented by vertical brackets. At least 10 mice were used in each experiment. An asterisk (*) indicates values significantly different from the ethanol control ($p < 0.05$). ○ salsolinol (920 μM/kg); ● 3-carboxysalsolinol (60 μM/kg); □ ethanol (87 mM/kg) + saline; ■ ethanol + salsolinol (60, 460, 920 μM/kg, from left to right, respectively); △, ethanol + 3-carboxysalsolinol (7.5, 15, 30, 60 μM/kg, from left to right, respectively).

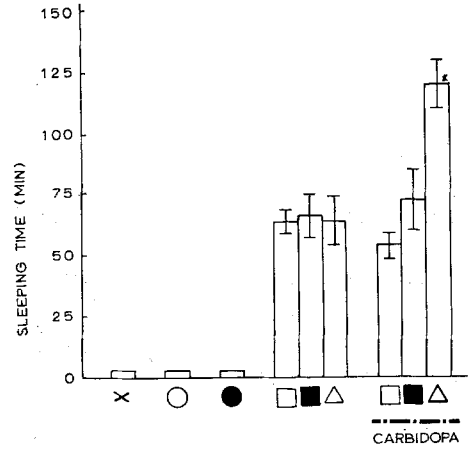

Fig. 3. Potentiation of ethanol-induced narcosis in mice by salsolinol and 3-carboxysalsolinol with and without carbidopa pretreatment. Representation of standard errors of means and the number of subjects used are the same as mentioned in Figure 2. An asterisk (*) indicates a value significantly different from its non-pretreated control ($p < 0.05$). X carbidopa (120 μM/kg); ○ salsolinol (60 μM/kg); ● 3-carboxysalsolinol (7.5 μM/kg); □ ethanol (87 mM/kg) + saline; ■ ethanol + salsolinol (60 μM/kg); △ ethanol + 3-carboxysalsolinol (7.5 μM/kg).

repeated with a pretreatment of carbidopa (120 μM/kg), a peripheral L-amino-acid decarboxylase inhibitor[15,16], administered orally as a suspension in 5% gum acacia, 60 min before the other drug treatments.

The onset of sleep did not vary significantly among the different experiments, but differences were observed in the duration of sleeping time. The effects of various isoquinoline treatments on ethanol-induced narcosis are illustrated in Figure 2. Neither salsolinol (60 μM/kg, 460 μM/kg, 920 μM/kg) nor 3-carboxysalsolinol (7.5 μM/kg, 15 μM/kg, 30 μM/kg, 60 μM/kg) produced any appreciable narcosis when administered alone. Ethanol led to loss of the righting reflex for approximately 1 h (64.3 $\pm$ 3.7 min). When applied in the lowest dose (60 μM/kg) the combination of salsolinol with ethanol produced no prolongation, but at the higher doses (460 μM/kg and 920 μM/kg) sleeping times were significantly prolonged ($p < 0.01$ and $p < 0.005$, respectively). The 3-carboxy analogue (60 μM/kg) significantly protracted the ethanol-induced narcosis ($p < 0.001$). Significant potentiation ($p < 0.025$) was also observed with two lower doses (30 μM/kg and 15 μM/kg) but not with the lowest dose (7.5 μM/kg).

The results demonstrate clearly that 3-carboxysalsolinol is a much more potent potentiator than salsolinol. Because a 30 or a 15 μM/kg dose of 3-carboxysalsolinol produces the same order of potentiation as a 460 μM/kg dose of salsolinol, there appears to be at least a 15-fold difference in potency.

The reason for the much higher potency of the 3-carboxysalsolinol is not definitely known at the present time, but an hypothesis can be offered. It is possible that the amino-acid compound, the 3-carboxy analogue, gains access to the central nervous system (CNS) more readily than the non-carboxylated compound, salsolinol – a relation similar to that of L-DOPA and dopamine[17]. Interaction with ethanol could then occur.

In reviewing the data presented above, it would seem that peripheral formation of salsolinol as a prime mechanism for the potentiation of ethanol sleeping time by dopamine, observed by ourselves[18] and other investigators[3], is unlikely in view of the relatively high doses of salsolinol required. It is noteworthy, however, that salsolinol (460 μM/kg) may still be a more effective potentiator than either DOPET (5.2 mM/kg) or tryptophol (1.5 mM/kg), the reduced metabolites[3,5].

The finding that ethanol sleeping time is potentiated by L-DOPA[4,18] may be the result of peripheral formation of 3-carboxysalsolinol. The high order of potency of the carboxylated compound suggests that the condensation of L-DOPA with acetaldehyde to form 3-carboxysalsolinol may be of importance in potentiating ethanol narcosis when L-DOPA is co-administered.

L-DOPA is known to undergo extensive metabolism in vivo, a factor that has led to the combined use of L-DOPA and peripheral decarboxylase inhibitors in the treatment of Parkinsonian patients[19]. To investigate the possibility that 3-carboxysalsolinol might also be decarboxylated peripherally, further studies which incorporated pretreatment with carbidopa were done. As indicated in Figure 3, carbidopa (120 μM/kg), 3-carboxysalsolinol (7.5 μM/kg) and salsolinol (60 μM/kg) did not cause any loss of righting reflex. In addition, none of these three compounds, in the doses used, enhanced the ethanol-induced narcosis (Figures 2 and 3). The 3-carboxy analogue, however, greatly prolonged ($p < 0.001$) ethanol sleeping time following the carbidopa suppression of peripheral decarboxylase, whereas the result with salsolinol was unchanged. This suggests that peripheral decarboxylation of 3-carboxysalsolinol may occur and that enzyme inhibition permits more of the compound to be available for penetration into the CNS. Upon reaching a central location its action of enhancing the ethanol-induced narcosis may be due to a depressant effect of the 3-carboxy analogue itself or of salsolinol which may be produced by decarboxylation in the CNS.

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Antitumor Activity of the Isodon Diterpenoids: Structural Requirements for the Activity

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Summary. A significant antitumor activity of oridonin (**1**) and lasiokaurin (**2**), the kaurene-type diterpenoids of *Isodon* species, was shown by their i.p. injection to the test mice inoculated by Ehrlich ascites carcinoma. Enmein (**8**), compounds **9** and **3** were also active under larger dose. Subsequently, the relationship between their chemical structure and antitumor activity was investigated, and the activity of oridonin (**1**) and lasiokaurin (**2**) was rationalized in terms of their structural feature.

Isodon japonicus Hara and *I. trichocarpus* Kudo (Labiatae) have been used as a home remedy in Japan. Previously, it was reported that a crude crystalline substance obtained from these plants showed an antitumor activity[2].

Recently, many kaurene and B-seco-kaurene type diterpenoids were isolated from these and other *Isodon* plants, and their structures were clarified[3]. Most of these diterpenoids contain an α-methylene cyclopentanone system in their molecule. We expected that this conjugated system would contribute to the activity, if the diterpenoids are active. Then, the antitumor activity of ori-

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