

in the different groups were consistent with the changes shown in Table 1. Thus, we found decreases of 48.4% ( $P < 0.001$ ) in group CNS, 9.8% (not significant) in group BP, 51.8% ( $P < 0.001$ ) in group BPL, 0.7% (not significant) in group Ab, 38.4% ( $P < 0.02$ ) in group AbL, 11.1% (not significant) in group Py and 37.1% ( $P < 0.02$ ) in group PyL, (control value:  $25.0 \pm 1.2 \mu\text{mol}$  glycolipid hexose per 100 mg of total lipid).

Our observations indicate that the presence of PBP in the injection mixture, necessary to induce the neurological disturbances observed in EAE, is not specifically required to induce the lipid alterations in the CNS. On this basis, EAE classically induced with whole CNS seems to be a composite result of different effects: the phenomena related to the paralytic symptoms elicited by the PBP, and the alterations induced by the lipidic components present in the CNS tissue, independent of clinical symptoms. Such differences between EAE induced by whole CNS and by PBP have already been observed. A demyelinating, or myelination inhibition, factor is present in serum of EAE animals sensitised with whole CNS but it is not consistently induced when PBP is used as encephalitogen<sup>8,9</sup>. The antibody to cerebroside is responsible both for the disturbances in the sulphatide metabolism and the demyelination effects observed in cord tissue cultures exposed to EAE serum<sup>8</sup>. Thus, it seems likely that our observations *in vivo* may be caused by similar phenomena, as suggested<sup>6</sup>. Inoculation of rabbits with cerebroside, adjuvant and albumin resulted in perivascular infiltration in the spinal cord but not paralysis<sup>10</sup>; these findings seem to be closely related to those recently observed in EAE.

On the other hand, the surprising results obtained with poly-L-lysine suggest that the basic nature of the protein injected is closely related to changes in the content of anionic lipids. This should lead to a study of the effects of the injection of other basic, acidic and neutral proteins on the lipid composition of the CNS. Recent findings<sup>11-14</sup> regarding the highly specific interaction of the myelin basic protein molecule with negatively charged lipids such as sulphatides at the air-water interface are relevant. If such interactions and molecular arrangements are also occurring *in vivo* in the CNS, the alterations observed may be interpreted as the consequence of specific disruptive responses directed towards some of the interacting molecules or particular structural assemblies.

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## Marijuana, absinthe and the central nervous system

THERE are striking similarities between the psychological actions of the liqueur absinthe<sup>1</sup> and the experiences frequently reported by users of marijuana<sup>2</sup>. We have therefore compared the properties of thujone and tetrahydrocannabinol (THC), which are believed to be the active principles of *Artemisia absinthium* and *Cannabis sativa*, respectively. Both substances are terpenoid, derived from the essential oils absinthol and cannabinol, and are formed by similar biosynthetic mechanisms<sup>3,4</sup>.

The effects of absinthe have been known since the last century, but thujone has been conspicuously absent from recent lists of psychotropic plant products. It has been traditionally grouped with two  $\text{C}_{10}\text{H}_{16}\text{O}$  isomers, camphor and menthol<sup>5</sup>, and classified as a convulsant poison. The molecular geometry of these three compounds is so different, however, that it is difficult to believe that, at low doses, they interact specifically with the same pharmacological receptors. At large doses, it is always possible that they exert similar, less specific actions by virtue of common physicochemical properties.

Thujone and THC have similar molecular geometry and similar functional groups available for metabolism. This close geometrical resemblance is illustrated in Fig. 1, in which the bonds common to both molecules are drawn as bold lines. The similarities include the gem-dimethyl groups at  $\text{C}_8$ , the  $\text{C}_7$  methyl groups, the bonds connecting carbons 8, 4, 3, 2 and 1 of THC and 8, 5, 4, 3 and 2 of thujone, the  $\alpha$  hydrogen at  $\text{C}_4$  in THC and the cyclopropyl 5-6 bond of thujone and the 4-5 bond in THC and the 1-5 bond in thujone. Finally, although there is

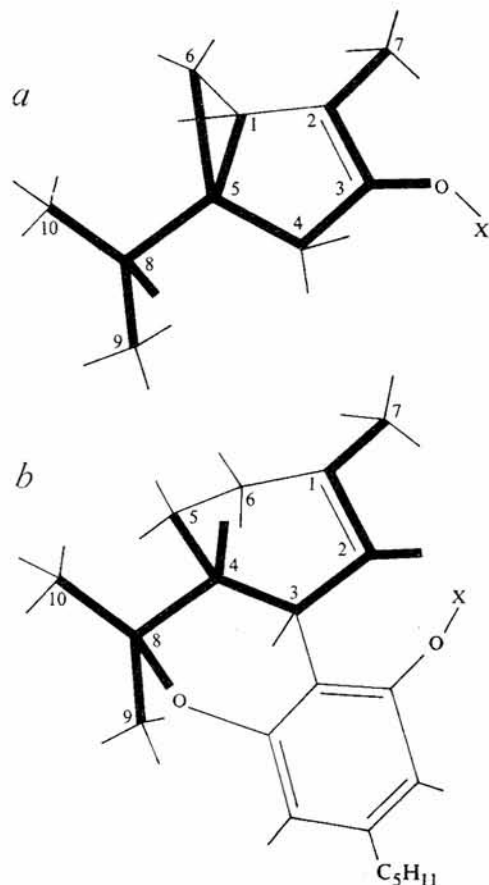


Fig. 1 Structural formulae of thujone-enol (a) and  $\Delta^1\cdot^6$ -THC (b). Bonds common to both molecules are drawn as bold lines. X indicates the site of the receptor with which the oxygen of the thujone molecule or the hydroxyl group of the THC molecule may react. See text for details.

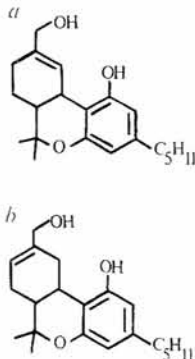


Fig. 2 Products of metabolism of  $\Delta^{1,2}$ -THC (a) and  $\Delta^{1,6}$ -THC (b).

no direct correspondence between the oxygen of the thujone molecule and the hydroxyl group of THC, it seems possible that both react with a common site of a pharmacological receptor, such as that indicated by X in Fig. 1, without changing the orientation or relative position of either molecule.

These oxygen atoms are likely to be the principal pharmacological binding sites because the primary metabolites from  $\Delta^{1,2}$ -THC (ref. 6) and  $\Delta^{1,6}$ -THC (ref. 7), both of which are physiologically active, are products of oxidation at C<sub>7</sub> (Fig. 2). Indeed, these metabolites have been suggested as the actual psychotomimetic agents in marijuana<sup>7</sup>.

The  $\Delta^{2,3}$ -enolic form of thujone contains an allylic group at C<sub>3,2,7</sub> which is analogous to C<sub>2,1,7</sub> in  $\Delta^{1,2}$ -THC; both systems should be available for similar oxidative reactions. In fact the electron-releasing ability of oxygen in the thujone enol should increase the rate of hydrogen removal at C<sub>7</sub> relative to a comparable reaction in THC (ref. 8). That thujone can exist in the  $\Delta^{2,3}$ -enol form is shown by its reaction with permanganate, a process known to involve enols<sup>9</sup>. Since the reaction affords an almost quantitative yield of the keto-acid<sup>10</sup> shown in Fig. 3 there is little doubt that the  $\Delta^{2,3}$ -enol form is involved. Further, the existence of enols in biological systems is well documented<sup>11</sup>.

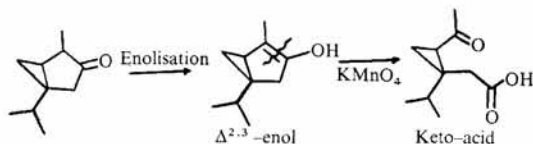


Fig. 3 Permanganate oxidation of thujone by way of the  $\Delta^{2,3}$ -enol.

We propose therefore that both thujone and THC exert their psychotomimetic effects by interacting with a common receptor in the central nervous system. Topologically, this receptor should have a binding site for interaction with oxygen, a planar region to accommodate the allyl system, and pockets or cavities in which the alkyl and hydrogen substituents common to both drugs would fit. This hypothesis suggests new experimental approaches to study the pharmacology and toxicology of these and related compounds. A common mechanism of action for THC and thujone is also interesting from a historical and sociological point of view.

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## Decrease in 17 $\beta$ -oestradiol receptor in brain of ageing rats

WE have shown<sup>1,2</sup> that the administration of 17 $\beta$ -oestradiol to ovariectomised female rats induces acetylcholinesterase (AChE; EC 3.1.1.7) in the cerebral hemisphere and cerebellum, and that this induction decreases with increasing age. The greatest induction occurs in the immature rat, and there is no induction in the old. As 17 $\beta$ -oestradiol receptors are present in rat brain<sup>3,4</sup>, we suggested that the impairment of AChE induction in the brain of old rats may be due to a decrease in the level of this receptor. This study was designed to test our hypothesis, and the data show for the first time that it may be correct.

Immature (7 week), adult (44 week) and old (108 week) female Wistar rats maintained in the rat colony were used. The rats of the three age groups were bilaterally ovariectomised and reared on tap water and standard diet for 21 d to ensure complete disappearance of oestradiol from the blood as described earlier<sup>1,2</sup>. They were killed on day 22 and the cerebral hemispheres were removed to prepare a 10% homogenate (w/v) in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2.8 mmol glucose.

The procedure followed for characterising the oestradiol-receptor complex of the cytosol was adopted from that of Wong and Burton<sup>5</sup> for the hydrocortisone-receptor in the nuclei of placenta. <sup>3</sup>H-oestradiol (0.2  $\mu$ Ci) (Radiochemical Centre, Amersham) was added to 1.0 ml aliquots of homogenate and the samples were kept on ice for 30 min with occasional stirring. They were then incubated at 37°C for 10 min in a water bath shaker after which 5.0 ml of  $1.5 \times 10^{-3}$  M MgCl<sub>2</sub> were added to each. Then they were kept in ice for 15 min and centrifuged at 700g for 15 min at 0°C. The pellet was resuspended in an equal volume of MgCl<sub>2</sub> and centrifuged as before. Both the supernatants were pooled and centrifuged at 14,500g for 1 h. The supernatant thus obtained was fractionated through a 11  $\times$  180 mm column of Sephadex G-25-80 (Sigma) at 2°C using an elution buffer of 0.6 M KCl, 10 mmol Tris and 1.5 mmol EDTA, pH 8.0. Fractions (1.0 ml) were collected and a 0.1 ml aliquot of each fraction was applied on 2  $\times$  2 mm Whatman No. 1 filter and dipped in 12.0 ml of scintillation fluid (PPO: POPOP; 4:0.4 in 1.0 l toluene). The radioactivity was counted in a Packard Tri-carb liquid scintillation spectrometer (Model 3003). The concentration of protein ( $\mu$ g ml<sup>-1</sup>) was measured according to the method of Sutherland *et al.*<sup>6</sup>. The averages of the data collected from four or five rats of each age are given in Fig. 1.

Figure 1 shows a sharp peak and a high level of oestradiol-protein complex in the cytosol of the immature rat as compared to those of the adult and the old rat. The peak for the radioactivity corresponds to the protein peak. It is also noteworthy that the peak for the labelled-complex gets diffused in the adult and the old indicating a loss of specificity or affinity of the receptor protein of the cerebral hemisphere which binds to oestradiol.

So far, the only enzyme of the cerebral hemisphere which