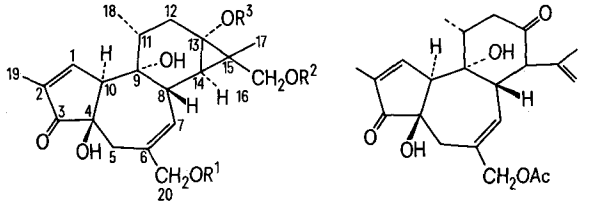
the form of two aliphatic esters. The candletoxins A and B represent the first aromatic derivatives of this parent diterpene to be isolated from natural sources.

Candletoxin A (**1**) was a glassy resin, hR_f 71 (Kieselgur G, 750 µm, coated with digol by developing their full length in 20% digol in acetone and air drying before use, solvent 25% ethylacetate in cyclohexane). Spectral data suggested that it was a triester of 12-deoxy-16-hydroxy-phorbol; IR (solid film KBr discs) V_{max}, 3420, 1730, 1630, 1605 cm^{-1}; CD (methanol), 335 nm ($\Delta\epsilon = -1.0$); 274 nm ($\Delta\epsilon = -0.67$); 227 nm ($\Delta\epsilon = +14.03$); 205 nm ($\Delta\epsilon = -16.70$): MS, an $M^{+\cdot}$ ion at m/e 608.2971 ($C_{35}H_{44}O_9$, error −2.5%) and fragment ions at m/e 590 (0.2%); 548 (2.0%); 530 (0.2%); 506 (2.5%); 499 (3.0%); 472 (5%); 446 (11%); 430(7%); 412 (10%); 394 (5%); 388 (5%); 370 (15%); 357 (10%); 352 (12%); 328 (15%); 310 (35%); 292 (10%); 282 (5%); 241 (12%); 223 (15%); 208 (35%); 179 (40%); 168 (25%); 161 (15%); 121 (45%); 109 (30%); 91 (100%): NMR-spectrum (CCl_4, 100 MHz) δ 7.51 b s 1H; δ 7.23 s 5H; δ 5.60 d (J = 4.8 Hz) 1H; δ 4.38 s 2H; δ 3.90 q (AB, J = 11 Hz) 2H; δ 3.98 s 2H; δ 3.20 m 1H; δ 3.08 m 1H; δ 2.54 m 1H; δ 2.34 b s 2H; δ 2.00 s 3H; δ 1.77 dd (J = 1.5 Hz) 3H; δ 1.54 m 2H; δ 1.26 s 3H; δ 1.16 d (J = 6 Hz) 3H; δ 0.93 complex 7H; δ 2.03 and δ 5.32 2 OH (deuterium exchange) ppm. The splitting of the allylic 2H signal of C(16) as a quartet can be understood by the proximity of the aromatic ring at C(13) (figure). Acid catalyzed transesterification of candletoxin A (**1**) (1% $HClO_4$ in CH_3OH) resulted in the production of candletoxin B (**2**) the C-20 hydroxy diester.



Candletoxin B (**2**) was also resinous, hR_f 24 in the same TLC system as before. This compound exhibited the following spectral data: IR V_{max} at 3420, 1730 (broad); 1630; 1605 cm^{-1}; C.D. (methanol), 337 nm ($\Delta\epsilon = -0.40$); 269 nm ($\Delta\epsilon = -0.8$); 227 nm ($\Delta\epsilon = +21.16$); 202 nm ($\Delta\epsilon = -24.60$); MS an $M^{+\cdot}$ ion at m/e 566.2859 ($C_{33}H_{42}O_9$, error −3.7%) and fragment ions at m/e 548 (4%); 530 (2%); 475 (1.5%); 463 (3%); 457 (12%); 446 (4%); 430 (12%); 412 (24%); 394 (16%); 373 (4%); 355 (8%); 346 (20%); 337 (4%); 328 (80%); 310 (84%); 292 (28%); 241 (40%); 223 (48%); 208 (88%); 179 (80%); 168 (68%); 161 (64%); 121 (88%); 120 (92%); 109 (84%); 95 (64%); 91 (100%); NMR-spectrum ($CDCl_3$ 100 MH_z), δ 7.55 b s 1H; δ 7.24 s 5H; δ 5.59 d (J = 5 Hz) 1H; δ 3.98 s 2H; δ 3.95 q (AB, J = 11 Hz) 2H; δ 3.58 s 2H; δ 3.26 m 1H; δ 3.02 m 1H; δ 2.47 b s 2H; δ 2.38 m 1H; δ 1.77 dd (J = 1.6 Hz) 3H; δ 1.53 m 2H; δ 1.25 s 3H; δ 1.16 d (J = 6 Hz) 3H; δ 0.93 complex 7H; δ 5.32, δ 2.17 and δ 1.5 3 OH (deuterium exchange) ppm. The absence of a 3H singlet at about δ 2.00 ppm and the diamagnetic shift of the 2H singlet of the C-20 position from δ 3.98 in **1** to δ 3.58 in **2** suggested that the C(20) acetyl moiety was absent in candletoxin B. Alkaline hydrolysis of **2** (0.5 M KOH in CH_3OH) produced a tetrol **3** which was converted to a diacetate **4**. (Acetic anhydride in pyridine (4:1).) MS an $M^{+\cdot}$ ion at m/e 566 (0.5%) and significant fragment ions at m/e 506 (2.5%); 426 (10%); 430 (5%); 310 (50%). The NMR-spectrum was similar to candletoxin (A) with the exception that signals due to the protons of α-methyl-butyrate were absent and an extra 3H singlet was exhibited at δ 2.01 ppm, thereby confirming the position of α-methyl-butyrate as C(16) in **1** and **2**.

Candletoxin B (**2**) was synthesized from the tetrol **3** by reaction with α-methyl-butyric anhydride in pyridine followed by acid catalyzed transesterification. Acetylation of **2** produced candletoxin A (**1**), thereby confirming that the phenylacetate moiety of **1** and **2** was present at C(13) of the tigliane nucleus. Complete hydrolysis of **1** and **2** (saturated barium hydroxide in methanol), followed by acetylation of the product produced a monoacetate which was recrystallized from acetone (m.p. 104–5 °C). The product **5** was identified as crotophorbolone monoacetate from its spectral data: IR $V_{max}^{CHCl_3}$ 3540; 3360; 1735; 1710; 1630 cm^{-1}: CD (CH_3OH), 210 nm ($\Delta\epsilon = -0.85$); 230 nm ($\Delta\epsilon = -4.76$); 273 nm ($\Delta\epsilon = +0.32$); 339 nm ($\Delta\epsilon = -0.40$); MS an $M^{+\cdot}$ ion at m/e 388 ($C_{22}H_{28}O_6$, 2%); 370 (2%); 328 (12%); 310 (17%); 292 (6%); 241 (21%); 208 (67%); 207 (81.5%); 179 (83.5%); 137 (33%); 122 (85%); 121 (77%); 91 (75%); 83 (100%). Base catalyzed elimination of the C(16) ester group with consequent formation of crotophorbolene confirms the nature of the parent diterpene as 12-deoxy-16-hydroxy-phorbol[6].

	R^1	R^2	R^3
1	CH_3CO	$CH_3 \cdot CH_2 \cdot CH(CH_3) \cdot CO$	$CO \cdot CH_2 \cdot$ 
2	H	$CH_3 \cdot CH_2 \cdot CH(CH_3) \cdot CO$	$CO \cdot CH_2 \cdot$
3	H	H	$CO \cdot CH_2 \cdot$
4	CH_3CO	CH_3CO	$CO \cdot CH_2 \cdot$

Crotophorbolone monoacetate (**5**)

Methiothepin and a 5-HT pathway to rat substantia nigra[1]

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Summary. Methiothepin reduced both median-raphe evoked and exogenous 5-HT depression of single substantia nigra neurones. While this is compatible with a serotonin releasing pathway, additional interactions of methiothepin with exogenous dopamine suggest the need for further pharmacological confirmation.

Recent evidence suggests the existence of a possible monosynaptic pathway from the median-raphe nucleus (MRN) to the substantia nigra (SN) of the rat[2,4]. Electrical stimulation of the MRN produces mainly depression of cell activity in the SN and this effect is well correlated with the response of the same neurones to electrophoretically administered 5-HT[4]. To establish further the identity of 5-HT as the inhibitory neurotransmitter we have

made pharmacological studies with the dibenzthiepin neuroleptic, methiothepin, an antagonist at central 5-HT receptors[5–9].

Experiments were performed in urethane anaesthetized (1.4 g kg^{-1} i.p.) adult rats (230–270 g) using standard electrophysiological techniques to record extracellular activity and to administer drugs by electrophoresis to spontaneously active SN neurones[3,4]. Aqueous drug solutions for electrophoresis were made up at pH 3.5–4.5 as follows: serotonin bimaleinate, dopamine hydrochloride (DA), GABA and acetylcholine chloride (ACh) were all 0.2 M; methiothepin was 4 mM (Hoffmann-La Roche & Co.). Submaximal stimulation of the MRN was performed as described previously and the recording micro-pipette tip-position was identified by the ejection of dye[3,4].

The effects of methiothepin were tested on 40 SN neurones whose activity was depressed during stimulation of the MRN and which responded reproducibly to electrophoretically administered 5-HT, DA, GABA or ACh. The concurrent ejection of methiothepin (5–75 nA, cationic current) for 5–13 min reduced depression (17/24 cells) but not excitation (0/8 cells) produced by 5-HT (figure 1A). In addition endogenous depression evoked by MRN stimulation was reversibly reduced in 22 of 40 cells (figure 1B). There was a close correlation between reduction of MRN evoked inhibition and reduction of 5-HT depressant responses by methiothepin (15/22 cells) on the same neurones. However, methiothepin also occassionally reduced the depressant (10/20 cells) or excitant (4/8) responses to DA, but rarely affected depression by GABA (2/24 cells) or excitation by ACh (1/17 cells). These interactions with methiothepin were sometimes accompanied by a gradual slowing of spontaneous neuronal firing (22/40 cells) but no obvious changes in spike amplitude. Most SN neurones (31) were found to be localized around the zona compacta-zona reticulata border. The rest were in the zona reticulata.

Previous electrophoretic studies have shown that methiothepin antagonized 5-HT but not GABA or DA depression of feline lateral geniculate neurones[9]. The present experiments in the rat SN confirm that methio-

Fig. 1A. Histogram of a spontaneous firing SN neurone (spikes sec^{-1} against minutes) showing reversible reduction of 5-HT inhibition produced by methiothepin. Drugs were ejected over a period indicated by the horizontal bar. Expelling currents are indicated in nA.

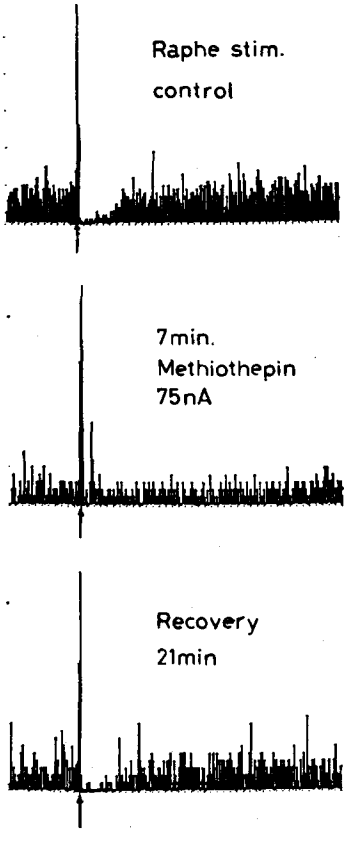

Fig. 1B. Computer generated post stimulus time histograms (200 sweeps; 250 msec sweep duration) showing raphe-evoked inhibition of a SN neurone. This was reversibly suppressed by methiothepin with an accompanying reduction in background firing rate. The position of the stimulus is indicated by the arrow above which is a stimulus artefact.

1 Acknowledgment. This work was supported by an MRC Programme Grant to Prof. D. W. Straughan.

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thepin may depress 5-HT-inhibitory but not excitatory responses. The correlation between the reduction of MRN-evoked inhibition and inhibition by exogenous 5-HT of the same SN neurones by methiothepin is compatible with a suggested MRN-SN serotoninergic pathway[4]. However, the additional interactions of methiothepin with DA observed in this and other studies[8] suggests the necessity for further pharmacological experiments to confirm the identity of 5-HT as the neurotransmitter in the MRN-SN pathway.

Liver changes following thiobenzamide poisoning

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Summary. Single thiobenzamide administration to rats induces liver necrosis. Chronic poisoning is followed by biliary cirrhosis. Areas of cholangiofibrosis are still evident after 4 months of recovery.

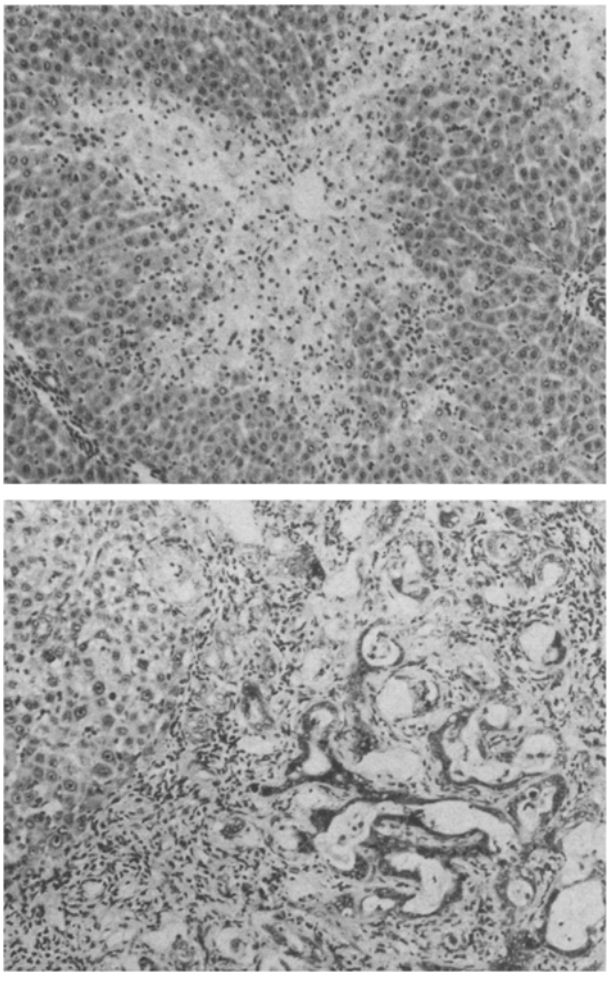
Thiobenzamide (TBA) (C_6H_5–$CSNH_2$) is an yellow, water-insoluble compound widely used as an intermediate for synthesis. It is also endowed with antibacterial activity against mycobacteria[1]. Even if there is little reason to add another compound to the ever-increasing list of those studied as able to cause liver damage, a toxicological study was undertaken for TBA in view of its structural similarity with the well-known toxic and carcinogen compound thioacetamide (TAA) (CH_3–$CSNH_2$). In fact some aspects of the mechanism of action of TAA are still debatable, in particular as regards the importance of the entire molecule or of an active group, the =S-moiety being strongly suspected[2].

The results reported here, showing that TBA causes a liver damage very similar to that due to the TAA, indicate that the lipid soluble TBA could be a useful tool in this field.

Methods. Male Sprague-Dawley rats bred in our colony were used. Acute intoxication was performed in suckling (7 days old) and adult rats starved for 12–16 h by i.p. administration of TBA suspended in 0.1% rat serum albumin at dose level of 30 mg/100 g; blocks of liver from 2 lobes were taken after 6, 12, 24, 48 and 120 h.

Chronic poisoning was obtained by feeding rats with the stock diet containing 0.050% TBA for a period of 4 months. The livers were examined after every month of intoxication, as well as after 4 additional months of normal diet. The liver tissue was fixed in Carnoy fluid and 10% buffered formalin. Frozen sections were stained with oil red for fat. Paraffin sections were stained with haematoxylin and eosin, PAS method with and without diastase for glycogen and Van Gieson method for connective tissue. Control rats (normal and TAA treated at equimolecular amounts) were used, with results identical to those reported by Gupta[3,4].

Results and discussion. Only the relevant changes observed in the liver will be reported and briefly discussed. Acute poisoning: 6 h after the TBA dose, centrolobular hepatocytes showed loss of cytoplasmic basophilia and glycogen and the mediolobular ones were filled with small fat droplets. The lobular distribution of liver damage was particularly evident after 24 h, when a central necrosis involved about one half of the lobule (figure, a), with a sharp boundary between the damaged hepatocytes and the periferal ones which retained their normal glycogen content, but had sligthly enlarged nuclei. The finding could be due to the incipient regeneration which was evident as a mitotic burst after 48 h, when the necrotic parts were filled with mononuclear inflammatory cells. 5 days later, the liver picture was indistinguishable from that of control animals.



a Centrolobular necrosis. *b* Area of cholangiofibrosis; hepatocytes on the left upper corner show prominent nucleoli.

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