Δ^1- and $\Delta^1(^6)$ Tetrahydrocannabinol[1]: Preliminary Observations on Similarities and Differences in Central Pharmacological Effects in the Cat

This report outlines data which show that although both Δ^1- and $\Delta^1(^6)$ tetrahydrocannabinol (Δ^1THC, $\Delta^1(^6)$ THC) slightly raise the reticular arousal threshold in the cat, they each induce different electroencephalographic responses.

Studies such as this have been made possible by the recent characterization of the active ingredients of *Cannabis sativa*[2,3]. However, even with this characterization, only 2 reports have been concerned with the effects of the tetrahydrocannabinols on reticular arousal – one describing a decreased threshold in the rabbit[4], and the other an increased threshold in the cat[5]. To our knowledge, although several reports have outlined the effects of cannabis extract and pure tetrahydrocannabinols on the electroencephalogram of various species[6–10] nothing has been reported concerning the differences induced by each of the Δ^1 and $\Delta^1(^6)$ THC isomers on the electroencephalogram in any species.

Method. 21 cats of either sex and ranging in weight from 2.4 to 3.9 kg were used in this study. The preparation of the acute animals has previously been detailed[11]. In each experiment a bipolar stainless steel electrode (24 gauge) insulated up to the tips was implanted into the mesencephalic reticular formation[12]. This electrode was used for eliciting reticular arousal as well as for recording reticular activity. Cortical activity was recorded by means of silver wires passed through hollow-centered screw guides which were screwed into the right occipital, sensorimotor and frontal areas. The reference screw guide was placed behind the tentorium cerebelli. The silver wires were insulated from the cortical screw guides by means of fine gauge polythene tubing. The electroencephalogram was recorded on an 8-channel, Type R, Offner Dynagraph. Reticular arousal was elicited by trains of pulses (300 Hz., 1 msec., 3 sec., voltage dependent upon threshold) delivered from a Grass S4 stimulator through a SIU478A isolation unit. Peripheral arousal was elicited by trains of pulses (10 Hz., 1 msec., 5 sec., voltage dependent upon threshold) delivered to a Palmer electrode placed on the femoral nerve. Control reticular and peripheral arousal thresholds were obtained over a one to two hour period, followed by the THC administration (1 dose per experiment) and monitoring of effects until their disappearance. The Δ^1 and $\Delta^1(^6)$ THC isomers, dissolved in 20% dimethylsulfoxide (DMSO), were administered i.v. in doses of 2 and 20 mg/kg. In one series, 10 mg/kg of each isomer was mixed and administered together. The DMSO did not alter electroencephalographic activity, reticular or peripheral arousal thresholds.

Results and discussion. In accordance with observations made in the cat[5], but contrary to those made in the rabbit[4], both Δ^1 and $\Delta^1(^6)$ THC raised the threshold to reticular induced arousal in our cat preparations. As outlined in the

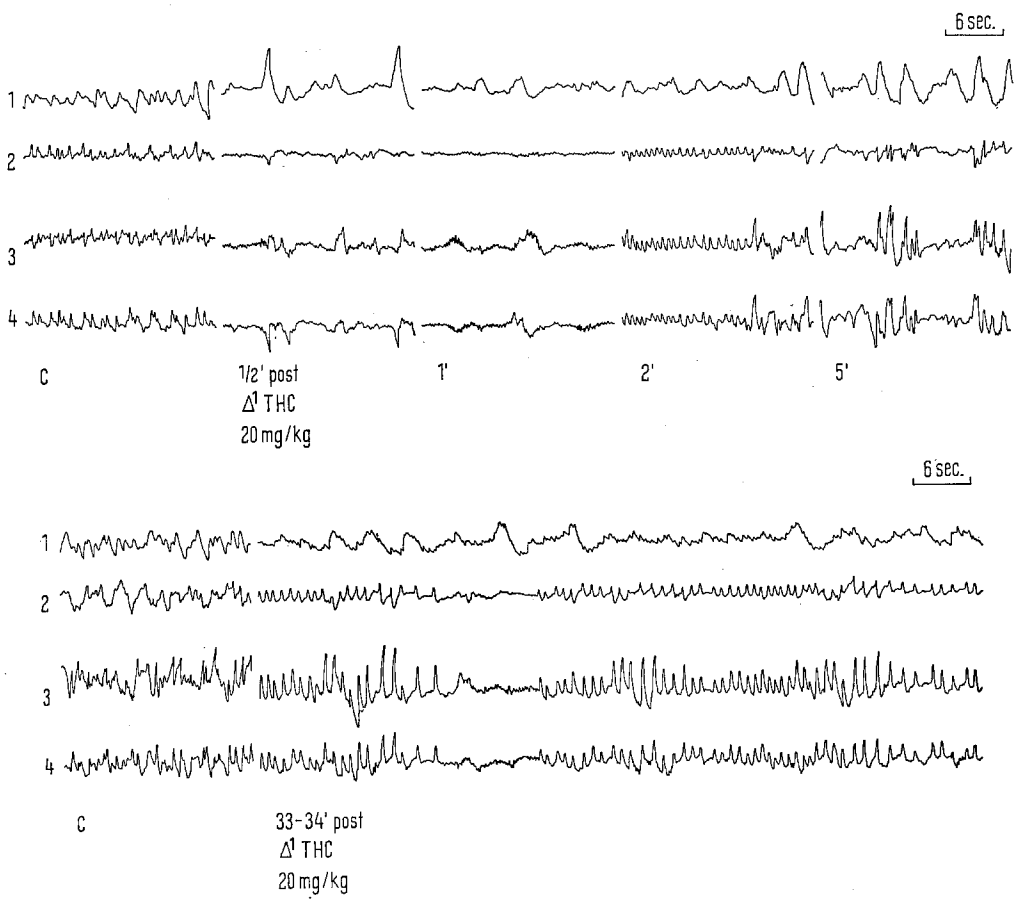

Fig. 1 a) and b). Electroencephalographic records taken at $^1/_2$, 1, 2, 5 and 33 to 34 min following the administration of 20 mg/kg Δ^1 THC. C = control. Channels: 1. mesencephalic reticular formation; 2. right occipital cortex; 3. right sensorimotor cortical area; 4. right frontal cortex.

Central effects of Δ^1 and $\Delta^{1(6)}$ THC in the cat

	Dose	No. of animals	Reticular arousal threshold (V)	Peripheral arousal threshold (V)	EEG alteration
Control	–	3	0.50 ± 0.05[a]	0.40 ± 0.04	Normal
20% DMSO	5 mls	3	0.50 ± 0.05	0.40 ± 0.04	Normal
Δ^1 THC	2 mg/kg	3	0.50 ± 0.05	0.40 ± 0.05	Normal
Δ^1 THC	20 mg/kg	3	0.90[b] ± 0.10	0.40 ± 0.04	+
$\Delta^{1(6)}$ THC	2 mg/kg	3	0.70[b] ± 0.07	0.40 ± 0.04	+
$\Delta^{1(6)}$ THC	20 mg/kg	3	0.70[b] ± 0.07	0.60[b] ± 0.06	+
Δ^1 THC+$\Delta^{1(6)}$ THC	10 mg/kg+10 mg/kg	3	0.50 ± 0.05	0.40 ± 0.04	+

[a] ± Standard deviation. [b] $P > 0.01$.

Table, single doses of both Δ^1 and $\Delta^{1(6)}$ THC which altered electroencephalographic activity, significantly raised the reticular arousal threshold, but did not have much, if any, effect on peripheral induced arousal. The higher dose of $\Delta^{1(6)}$ THC (20 mg/kg) raised the reticular arousal threshold to the same extent as the lower dose (2 mg/kg). The increase in reticular threshold induced by the higher dose of Δ^1 THC was similar to that induced by both doses of the $\Delta^{1(6)}$ isomer. Although the combined dose of both isomers (10 mg/kg of Δ^1 THC plus 10 mg/kg of $\Delta^{1(6)}$ THC) induced electroencephalographic change, it did not alter the reticular arousal threshold (Table).

Although the reticular arousal threshold was altered in a similar manner by both THC isomers, each induced different electroencephalographic responses. HOCKMAN et al.[10] have reported electroencephalographic changes with doses of Δ^1 THC ranging from 0.5 to 5 mg/kg. In our study, however, the lowest dose of Δ^1 THC used (2 mg/kg) did not alter the electroencephalogram. Although our results with the lower dose of Δ^1 THC do not confirm the results obtained by HOCKMAN et al.[10], and the reasons for this may be the different animal preparations used in the respective studies or differences in the potency of the THC isomers, no conclusions can be drawn before definitive studies are made of a more comparative nature.

The different electroencephalographic responses induced by the Δ^1 and $\Delta^{1(6)}$ THC isomers are represented in Figures 1–3. For purposes of clarity and reproducibility, representative records have been taken from animals which received the 20 mg/kg dose of each isomer. In agreement with the results of HOCKMAN et al.[10] Δ^1 THC induced a

[1] L. CROMBIE, *The Botany and Chemistry of Cannabis* (Eds. C. R. B. JOYCE and S. H. CURRY; J. and A. CHURCHILL, London 1970), p. 209.
[2] R. MECHOULAM, Science *168*, 1159 (1970).
[3] R. MECHOULAM, A. SHANI, B. YAGNITINSKY, Z. BEN-ZVI, P. BRAUN and Y. GAONI, *The Botany and Chemistry of Cannabis* (Eds. C. R. B. JOYCE and S. H. CURRY; J. and A. Churchill, London 1970), p. 93.
[4] H. I. BICHER and R. MECHOULAM, Archs int. Pharmacodyn. Thér. *172*, 24 (1968).
[5] E. F. DOMINO, H. F. HARDMAN and M. H. SEEVERS, Univ. Michigan med. Cent. J. *36*, 240 (1970).
[6] B. C. BOSE, A. Q. SAIFI and A. W. BHAGWAT, Archs int. Pharmacodyn. Thér. *147*, 285 (1964).
[7] J. MASUR and N. KHAZAN, Life Sci. *9*, 1275 (1970).
[8] E. A. RODIN, E. F. DOMINO and J. P. PORZAK, J. Am. med. Ass. *213*, 1300 (1970).
[9] E. F. DOMINO, Abstr. Marihuana Conference, N.Y. Acad. Sci., New York (1971).
[10] C. H. HOCKMAN, R. G. PERRIN and H. KALANT, Science *172*, 968 (1971).
[11] B. LECLERCQ and M. SEGAL, Can. J. Physiol. Pharmac. *43*, 491 (1965).
[12] H. H. JASPER and C. AJMONE-MARSON, *A Stereotaxic Atlas of the Diencephalon of the Cat* (The National Research Council of Canada, Ottawa).

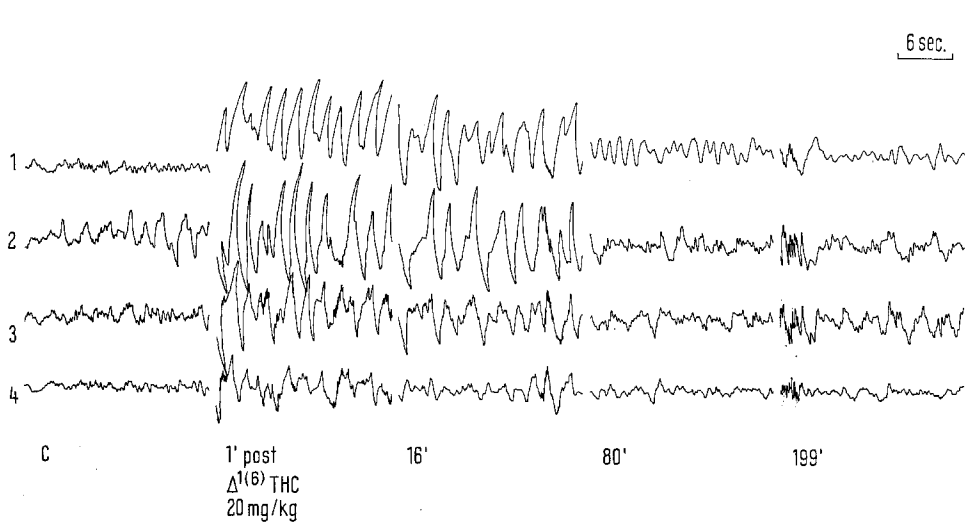

Fig. 2. Electroencephalographic records taken at 1, 16, 80 and 199 min following the administration of 20 mg/kg $\Delta^{1(6)}$ THC. C = control. Channels: 1. mesencephalic reticular formation; 2. right occipital cortex; 3. right sensorimotor cortical area; 4. right frontal cortex.

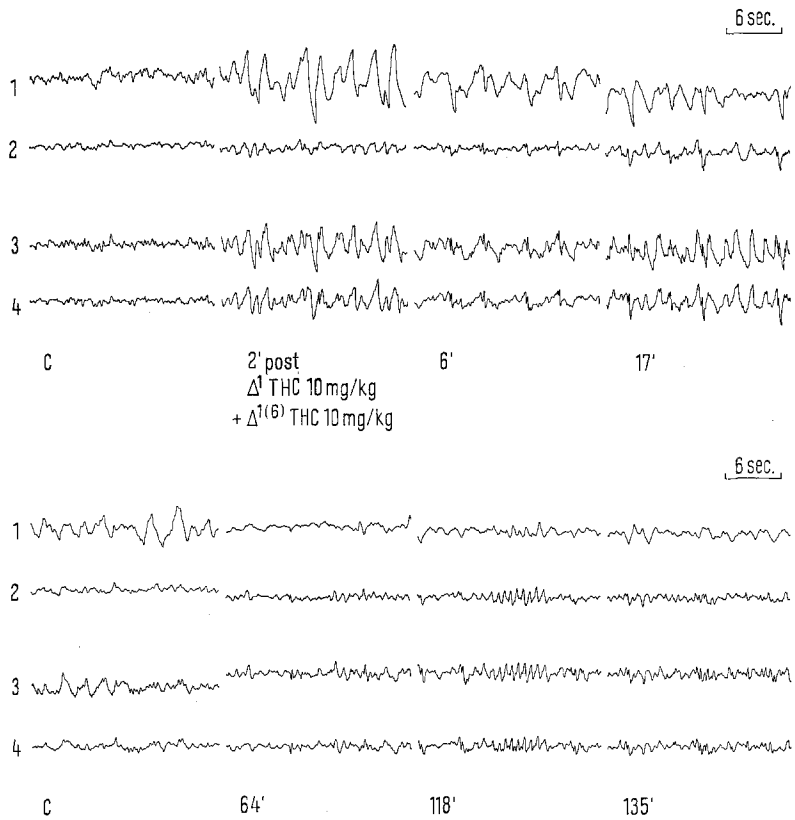

Fig. 3 a) and b). Electroencephalographic records taken at 2, 6, 17, 64, 118 and 135 min following administration of a mixture of 10 mg/kg each of Δ^1 and $\Delta^1(6)$ THC. C = control. Channels: 1. mesencephalic reticular formation; 2. right occipital cortex; 3. right sensorimotor cortical area; 4. right frontal cortex.

triphasic electroencephalographic response. As seen in Figures 1 a) and b), $^1/_2$ to 1 min following the administration of the 20 mg/kg dose, the electroencephalogram shows a marked arousal pattern (decreased voltage, increased frequency). At 2 min, one sees cortical synchronization followed 3 min later by high voltage spiking activity. These triphasic alterations continued throughout the duration of the overall effect which lasted approximately 6 h. The amplitude of the spikes, however, decreased at 4 h and continued to do so until the effect subsided. Figure 1 b) shows the arousal pattern interspersed with synchronous waxing and waning spike activity. The significance of this electroencephalographic response cannot be presently assessed in terms of behavioral changes produced by the 20 mg/kg dose of Δ^1 THC in the cat.

Contrary to the electroencephalographic response induced by Δ^1 THC in this study, and to that reported on cortical activity in the rabbit[4], both doses of $\Delta^1(6)$ THC (2 and 20 mg/kg) induced a marked electroencephalographic depression in the present study (Figure 2). 1 min following 20 mg/kg of $\Delta^1(6)$ THC, both the cortical and reticular activity show the marked slowing in frequency and increased voltage pattern indicative of a depressed state. The cortical activity reverted to normal within $1^1/_2$ h, whereas the reticular activity returned to normal in about $2^1/_2$ h. The total duration of effect of the 20 mg/kg dose of the $\Delta^1(6)$ THC isomer was much shorter than that of the Δ^1 THC isomer which had been previously administered in the same quantity.

Figures 3 a) and b) show the effect of a combined dose of 10 mg/kg of each isomer. As can be seen, within 2 min of administration, there is a marked slowing in the frequency of both the cortical and reticular activity along with a marked increase in voltage. Although this effect is qualitatively similar to the effect obtained with the 20 mg/kg dose of $\Delta^1(6)$ THC, it is not quantitatively as large. Approximately 1 h later (Figure 3 b), 64 min), when the effect of the $\Delta^1(6)$ isomer has subsided (as already outlined above and in Figure 2), we observed the arousal pattern attributable to the Δ^1 THC isomer. In addition, synchrony resembling that induced by the Δ^1 THC isomer was observed in this experiment, but only 118 min after both isomers had been administered and at a time when the $\Delta^1(6)$ THC effect has apparently subsided. At this dose ratio (i.e. 50/50 of each of Δ^1 and $\Delta^1(6)$ THC), it appears that the effects of the $\Delta^1(6)$ THC isomer predominate during the first hour.

We realize that the doses of Δ^1 and $\Delta^1(6)$ THC used in this study are well above the doses used by HOCKMAN et al.[10] and in excess of the amounts used in human studies[8, 13, 14] to induce mild to hallucinogenic effects. However, it is only at these larger pharmacological doses that these differences are observed.

The differences between our results in the cat and those of BICHER and MECHOULAM[4] in the rabbit could be accountable on the basis of a species difference. Since both THC isomers are reportedly converted to an active hydroxy-metabolite[15–18], it would be of great interest to de-

[13] A. T. WEIL, N. E. ZINBERG and J. M. NELSON, Science *162*, 1234 (1968).
[14] H. ISBELL, C. W. GORODETZSKY, D. JASINSKI, U. CLAUSSEN, F. v. SPULAK and F. KORTE, Psychopharmacologia *11*, 184 (1967).
[15] R. L. FOLTZ, A. F. FENTIMAN JR., E. G. LEIGHTY, J. L. WALTER, H. R. DREWES, W. E. SCHWARTZ, T. F. PAGE and E. B. TRUITT, Science *168*, 844 (1969).
[16] I. M. NILSSON, S. AGURELL, J. L. G. NILSSON, A. OHLSSON, F. SANDBERG and M. WAHLQVIST, Science *168*, 1228 (1970).
[17] S. AGURELL, I. M. NILSSON, A. OHLSSON and F. SANDBERG, Biochem. Pharmac. *19*, 1333 (1970).
[18] M. E. WALL, Abstr. Marihuana Conference, N. Y. Acad. Sci., New York (1971).

termine which THC isomer, if any, the hydroxy-metabolite simultates in its electroencephalographic effects.

LERNER and ZEFFERT[19] reported that the ratio of $\Delta^1(^6)$ THC/Δ^1 THC found in fresh Mexican marihuana was 0.4/99.6, whereas in older samples of hashish[19] and in Maryland marihuana[20] the $\Delta^1(^6)$ THC isomer represented 10% of the active THC fraction. In the light of this evidence and the different electroencephalographic responses induced by the Δ^1 and $\Delta^1(^6)$ isomers, the ratio of Δ^1 to $\Delta^1(^6)$ THC may be a necessary factor to take into account when assessing the behavioral, pharmacological and clinical effects of marihuana in animals and man[21].

Bien que le Δ^1 ainsi que le $\Delta^1(^6)$ tétrahydrocannabinol élevent le seuil de la formation réticulaire chez le chat, les réponses électroencéphalographiques fournies par ces composés sont distinctes. Il faut considérer ces différences dans l'évaluation pharmacologique et clinique des effets de marihuana chez l'homme et chez des animaux.

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Department of Pharmacology, Dalhousie University, Halifax (Nova Scotia, Canada), 8 November 1971.

[19] M. LERNER and J. T. ZEFFERT, Bull Narcot. *20*, 53 (1967).
[20] R. L. HIVELY, W. A. MOSHER and F. W. HOFFMAN, J. Am. chem. Soc. *88*, 1832 (1966).
[21] Samples of Δ^1 and $\Delta^1(^6)$ THC were supplied by Professor R. MECHOULAM, Laboratory of Natural Products, The Hebrew University of Jerusalem (Israel), and by the Federal Food and Drug Laboratories, Ottawa (Canada.)

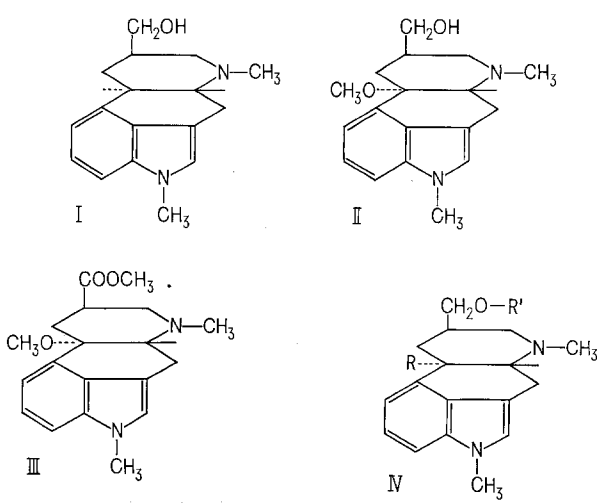
10-Methoxyergoline Derivatives as α-Adrenergic Blocking Agents[1]

Natural ergot alkaloids possess various pharmacological actions and among them a strong alpha adrenergic blocking activity[2,3]. Hydrogenation of ergotamine and ergotoxine yields drugs with enhanced adrenolytic activity and reduced toxicity, however the specifity of action of these compounds is still unsatisfactory. For instance dihydroergotoxine, a combination of dihydroergocristine, dihydroergocryptine and dihydroergocornine in equal proportions, has a central blood pressure depressing activity and a latent vasoconstrictor action becoming manifest in spinal cats when the destruction of the medulla oblongata prevents its central hypotensive effect[4]. Furthermore in dogs, this drug is a powerful emetic agent at i.v. doses close to those found to block the alpha receptors[5].

Although modifications of the ergoline nucleus are known to alter the pharmacological profile of the ergot alkaloids[2], most of the compounds so far reported are substituted amides of lysergic acid. In order to obtain more specific pharmacological agents and, notably, a more specific alpha adrenergic blocking agent, we have subjected, in the last years, both the ergoline nucleus and the amide linkage to a series of chemical modifications[6,7]. In the course of this work we have examined various esters of 1-methyldihydrolysergol (I)[8] and, among them, the benzoate (No. 1) and the nicotinate (No. 2) were found to have a certain adrenolytic activity. The corresponding esters of 1-methyl-10α-metoxydihydrolysergol (II)[9] were next investigated (No. 3 and 4). The nicotinate (No. 4) was found to be remarkably active, whereas the picolinate (No. 5), the isonicotinate (No. 6) and 3-pyridylacetate (No. 7) were practically devoid of adrenolytic activity. On the basis of these results, a series of nicotinic esters bearing various substituents in the pyridine nucleus were synthesized. The physico-chemical data and the biological activities of the compounds IV so far synthesized are reported in the table[10].

Chemistry. Reduction of the ester III[11] with an excess of $LiAlH_4$ in tetrahydrofuran afforded 10 α-methoxydihydrolysergol, m.p. 225–227° (Anal. Calcd. for $C_{17}H_{22}N_2O_2$: C 71.3; H 7.7%. Found C 71.2; H 8.0%) which was methylated with CH_3I and KNH_2 in liq. NH_3[12] to give II, m.p. 212–214°, $[\alpha]_D^{20}$-10° (c 0.5, pyridine). The esters were prepared by condensation of II with an excess of acyl chloride in pyridine: comp. No. 19 was obtained by reaction of II (1 mol) with 5-carboxamidonicotinic acid[13] (2 mol) in pyridine, in the presence of dicyclohexylcarbodiimide (2 mol) (24 h, room temp)[14].

Pharmacology. The data reported in the Table show that among the pyridyl carboxylic esters of 1-methyl-10α-methoxydihydrolysergol, the nicotinates, and notably



[1] Ergoline derivatives. Note X, Note IX: W. BARBIERI, L. BERNARDI, G. BOSISIO and A. TEMPERILLI, Tetrahedron *25*, 2401 (1969).
[2] A. STOLL and A. HOFMANN in *The Alkaloids*, Vol. 7, (Ed. R. H. F. MANSKE; Academic Press New York 1965), p. 772.
[3] A. CERLETTI in *Neuropsychopharmacology* (Eds. P. B. BRADLEY, P. DENIKER and C. RADONCO-THOMAS; Elsevier Publ. Co. Amsterdam 1959) pag. 117.
[4] H. KONZETT and E. ROTHLIN, Br. J. Pharmac. *8*, 201 (1953).
[5] S. C. WANG and V. GLAVIANO, J. Pharmac. exp. Ther. *111*, 329 (1954).
[6] L. BERNARDI, Chimica Ind. Milano *51*, 563 (1969).
[7] G. B. FREGNAN and A. H. GLÄSSER, Experientia *24*, 150 (1968).
[8] L. BERNARDI and O. GOFFREDO, USP No. 3,236,852 (to Farmaceutici Italia).
[9] L. BERNARDI, G. BOSISIO and O. GOFFREDO, USP No. 3,228,943 (to Farmaceutici Italia).
[10] All the compounds gave satisfactory analysis (C, H, N).
[11] W. BARBIERI, L. BERNARDI, G. BOSISIO and A. TEMPERILLI, Tetrahedron *25*, 2401 (1969).
[12] F. TROXLER and A. HOFMANN, Helv. chim. Acta *40*, 1721 (1957).
[13] Swiss Pat. No. 284.073.
[14] We are indebted to Dr. P. ULIVI for this preparation.