Tetrahedron Letters No.41, pp. 3603-3606, 1969. Pergamon Press. Printed in Great Britain.

A QUATERNARY N-DIHYDROPYRROLIZINOMETHYL DERIVATIVE OF HELIOTRINE FROM *HELIOTROPIUM EUROPAEUM*

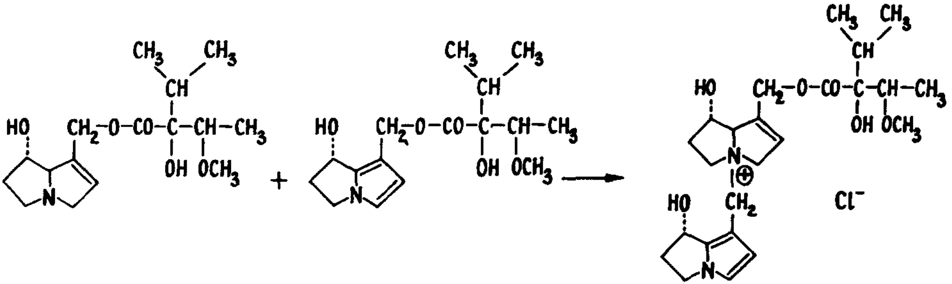
By C. C. J. Culvenor and L. W. Smith

Division of Applied Chemistry, C.S.I.R.O., Melbourne, Australia

(Received in UK 28 July 1969; accepted for publication 8 August 1969)

Some years ago, one of us reported the isolation of a minor constituent of the seed alkaloid of *Heliotropium europaeum* and assigned to it the formula $C_{12}H_{17}O_4N$ or $C_{12}H_{19}O_4N$ (1). The compound was isolated from a countercurrent distribution fraction consisting largely of heliotrine (I) and has not been detected in numerous other separations of the alkaloids of this species. Data accumulated since has shown that the compound is more complex than originally thought and its essential character was recognised during recent studies on the dihydropyrrolizine analogues of the pyrrolizidine alkaloids (2). The structure (II), regarded as the product of alkylation of a molecule of heliotrine by dehydroheliotrine (III), is now assigned to this substance. It appears to occur naturally in *H. europaeum* and thus provides the first evidence that the dihydropyrrolizine analogues are formed in plant species along with the known alkaloids.

The presence of chlorine was suspected when analyses for C, H, O and N did not add to 100%. The content of ionic chlorine was found to be 6.1% and the microanalyses then favoured the empirical formula $C_{24}H_{37}O_6N_2Cl$. (Found: C, 59.3; H, 7.8; N, 6.1; O, 20.3;



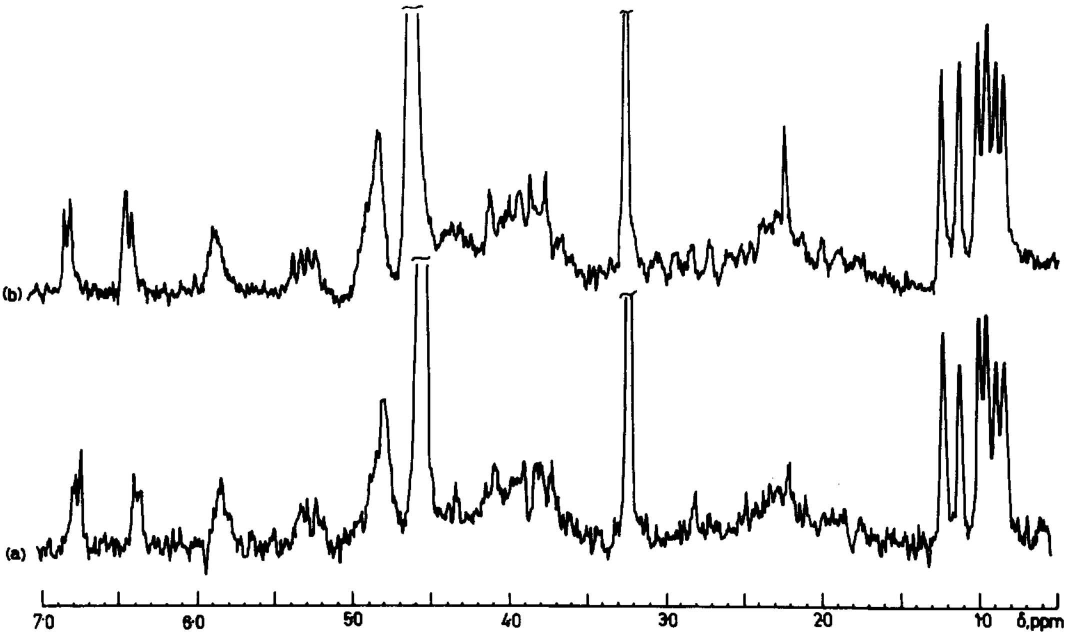
(I) (III) (II)

Cl, 6.1%. Calc. for $C_{24}H_{37}O_6N_2Cl$: C, 59.4; H, 7.7; N, 5.8; O, 19.8; Cl, 7.3%). This formula cannot be confirmed from the mass spectrum which is similar to that of heliotrine, $C_{16}H_{27}O_5N$, and contains no peak at higher mass number than 313, the molecular weight of heliotrine. Absorption in the ultraviolet is largely end absorption but includes a peak, λ_{max} 215 mμ, log ε 4.13, initially regarded as indicative of an αβ-unsaturated ester but similar also to the absorption of pyrrole derivatives (3). The compound is too sparingly soluble to give infra-red spectra in solution but a Nujol mull shows absorption at 3420 and 3160 cm^{-1} (OH or NH), 1715 cm^{-1} (unsaturated or hydrogen-bonded ester carbonyl), 1570 cm^{-1}, 880 cm^{-1} and 725 cm^{-1}. The n.m.r. spectrum (Fig.1a) shows the following signals appropriate to groupings present in the heliotrine moiety: two 3-proton doublets, δ 0.87, 0.93, due to $CH(\underline{CH_3})_2$, another 3-proton doublet, δ 1.15, and a 3-proton singlet, δ 3.21, due to $CH(O\underline{CH_3})\underline{CH_3}$, a 1-proton multiplet, δ 5.85, due to $\underline{CH} = C$ and a group of signals centred near δ 4.80, integrating for four protons of which two are considered to be $\underline{CH_2}OCOR$. The remaining signals of this group are now assigned to the methylene group located between the pyrrole ring and the quaternary nitrogen. The 1-proton multiplet, δ 5.27, resembles in appearance and in chemical shift the proton at C7 in diesters of heliotridine and retronecine but it is now assigned to the proton at C7 in the dehydroheliotrine moiety. In dehydroheliotrine, this proton gives rise to a quartet, δ 5.25. Although the two 1-proton doublets, δ 6.40, 6.78 (J 2.5 c/s) could possible be due to a grouping such as $\underline{CH_2}=C-CO_2R$, they also closely resemble the doublets, δ 6.21, 6.55, due to the H2 and H3 protons of the dihydro-5H-pyrrolizine ring in (III) (2). Since the compound gives an intense mauve colour with Ehrlich's reagent, the doublets can be definitely assigned to a pyrrole ring. In keeping with the quaternary structure (II), the background signals in the region δ 2.0-4.0 are at somewhat lower field than in most pyrrolizidine alkaloids (e.g. the broad envelope centred near δ 2.25 is usually centred near δ 1.9).

The structure which best accommodates this data is the dimeric molecule (II). Cleavage by zinc reduction to give heliotrine or by electron-impact to give a heliotrine ion and its further fragmentation products is readily explicable. Proof of the structure (II) for the compound has now been obtained by its preparation from heliotrine and dehydroheliotrine. The two reactants in dimethylsulphoxide at 50° gave in fair yield a product which, after replacement of the anion by chloride ion on Amberlite IRA400 resin, had the same

Figure 1

Nuclear magnetic resonance spectra measured in $CDCl_3$ at 60 Mc/s: (a) compound $C_{24}H_{37}O_6N_2Cl$ from *Heliotropium europaeum*, (b) product from heliotrine and dehydroheliotrine.



melting point, chromatographic, spectral and optical rotatory properties as the compound from *Heliotropium europaeum*. The n.m.r. spectra are compared in Fig.1.

The stability of (II) to acids and to storage at room temperature is considerably greater than that of other dihydropyrrolizine derivatives such as dehydroheliotrine, but there was doubt as to whether (II) occurs naturally in the seed of *Heliotropium europaeum*. The original source of (II) was a methanol extract of 70 lb of seed of *H. europaeum*, the extract being possibly evaporated in an iron still. Mattocks (4) has shown that N-oxides such as heliotrine N-oxide may be converted by ferrous ions into the corresponding dihydropyrrolizine (giving in this instance (III)). The same change may, of course, occur in the living plant. If (III) were produced in the presence of heliotrine, the formation of (II) would follow. We find that if (II) were present in the initial extract it could survive, at least in part, the zinc-sulphuric acid reduction and partitioning to which the extract was subjected (1). In particular (II) passes readily into 1:1 carbon tetrachloride-chloroform from saturated aqueous sodium bicarbonate, the phases used in the countercurrent distribution from which (II) was isolated. We have now shown that a substance with the chromatographic properties and Ehrlich colour reaction of (II) can be detected in extracts of fresh vegetative parts and in extracts of seed of *H. europaeum*.

REFERENCES

1. C. C. J. Culvenor, *Australian J. Chem.* 7, 287 (1954).
2. C. C. J. Culvenor, J. A. Edgar, L. W. Smith and H. J. Tweeddale, *Tetrahedron Letters* 1969, preceding paper.
3. A. E. Gillam and E. S. Stern, "Introduction to Electronic Absorption Spectroscopy in Organic Chemistry" (Edward Arnold, London), Second Edition, 1960, p.150.
4. A. R. Mattocks, *Nature* 219, 480 (1968).