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Electron Capture Gas Chromatography Detection and Mass Spectrum Identification of 3-(2'-Ketohexyl)indoline in Spinal Fluids of Patients with Tuberculous Meningitis

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A basic, extractable, indolic type of compound, which was derivatized with heptafluorobutyric anhydride and pyridine, was obtained from the cerebrospinal fluids of patients with acute tuberculous meningitis. The compound was detected by frequency-pulsed, modulated electron capture gas-liquid chromatography, and it was tentatively identified by mass spectrometry as 3-(2'-ketohexyl)indoline. The compound was found to be valuable for differentiating between tuberculous, cryptococcal, and aseptic meningitides.

Recent data reported from this laboratory (R. B. Craven et al., submitted for publication) showed that frequency-pulsed modulated electron capture (FPEC) gas-liquid chromatography (GLC) could be used to analyze electron-absorbing derivatives prepared from extracts of cerebrospinal fluids (CSF) and that the patterns obtained with fluids from patients with acute cases of tuberculous, aseptic, and cryptococcal meningitides differed (Fig. 1A, B, and C, respectively). These findings are important because they could provide the physician information that would permit early specific treatment of tuberculous and cryptococcal meningitides without having to wait several days for the causative agent to be identified by cultural methods.

A component that gave between one-fourth to full-scale deflection on the recorder was detected in the basic extractions of CSF taken from 12 patients with acute untreated tuberculous meningitis. This compound disappeared with effective therapy and was important as a marker for distinguishing between tuberculous, aseptic, and cryptococcal meningitides (Fig. 1A). Because of its importance as a marker and because it possessed amine characteristics, we decided to identify the compound by GLC-mass spectrometry.

MATERIALS AND METHODS

Reagents. Chloroform used in the study was of nanograde quality (Mallinckrodt). Heptafluorobutyric anhydride (HFBA) and melatonin (Pierce Chemicals) and tryptamine (Aldrich) were reagent grade. Spectrophotometric-grade pyridine (Mallinckrodt) was used. Ethanol was undenatured absolute (99.5%). 5-Methyltryptamine was obtained from Sigma Chemical Co.

Standards. A portion (20 μmol) of melatonin, tryptamine, and 5-methyltryptamine was added to 10 ml each of distilled water and permitted to sit at room temperature until the respective compounds dissolved. Then, a series of dilutions was made in water. Aqueous samples were extracted and derivatized as described below.

Preparation of derivatives. Two-milliliter samples of CSF taken from patients with acute tuberculous, aseptic, or cryptococcal meningitis along with aqueous standards were acidified to about pH 2 with 0.1 ml of 50% (vol/vol) H_2SO_4. The acidified samples were extracted by shaking vigorously with 20 ml of a chloroform-ethanol solution (94:6). The residual aqueous phase was then made basic (about pH 10) with 8 N NaOH and extracted again with 20 ml of chloroform. The basic extracts, which contained the amines, were derivatized with HFBA-pyridine-ethanol as previously described (1). The derivatized samples in chloroform were evaporated almost to dryness with clean, dry air, and 0.1 ml of a final solvent, consisting of either ethyl ether (for mass spectrometry) or xylene-ethanol (50:50), was added. Two microliters was used for FPEC GLC analysis, and 4 to 8 μl was used for GLC-mass spectrometry analysis.

Apparatus. Derivatives were analyzed on Perkin-Elmer 900 and 3920 gas chromatographs equipped with ^{63}Ni-labeled FPEC detectors and dual-glass columns (0.3-cm ID by 7.6-m length). The columns were packed with either 3% OV-1 or 3% OV-101 liquid-phase material coated on Chromosorb W 80/100 mesh. For analysis of the HFBA derivatives, the instrument was held isothermally at 90°C for 8 min, programmed for a linear increase of 4°C per min to 225°C, and held at 225°C for 24 min. Some HFBA derivatives of melatonin were analyzed isothermally at 125°C. A mixture of argon-methane (95:5)

was used as carrier gas at a flow rate of 50 ml/min. Flush gas was added to increase the flow at the detector to 67 ml/min. Detector temperature was set at 275°C. Standing current was set at 2, and attenuation was 512 for programmed analysis and 64 for isothermal analysis. A Beckman recorder was used with an input signal of 1 mV and a chart speed of 76.2 cm/h (0.5 inch/min).

Gas chromatography-mass spectrometry. An LKB model 9000 mass spectrometer equipped with a System Industry system 150 computer package and a Dupont model 21-491B mass spectrometer interfaced with a Varian 2700 gas chromatograph were used to obtain mass spectra. The 21-491B was equipped with chemical ionization potential, and the interfacing gas chromatograph contained an electron capture detector. Resolution of both instruments was approximately 1,000. The effluent from the LKB gas chromatograph was monitored by a total ion current detector. The gas chromatographs were equipped with glass columns (0.3-cm ID by 3.6 m in length) that were packed with Chromosorb W 80/100 mesh (AW-DMCS H.P.) coated with 3% OV-1. Helium was used as carrier gas, and methane was used as reagent gas for chemical ionization. The LKB gas chromatograph was programmed at 5°C per min from 90 to 225°C, and the Dupont gas chromatograph was programmed at 4°C per min from 90 to 225°C. Both instruments were then held isothermally at 225°C for 48 min.

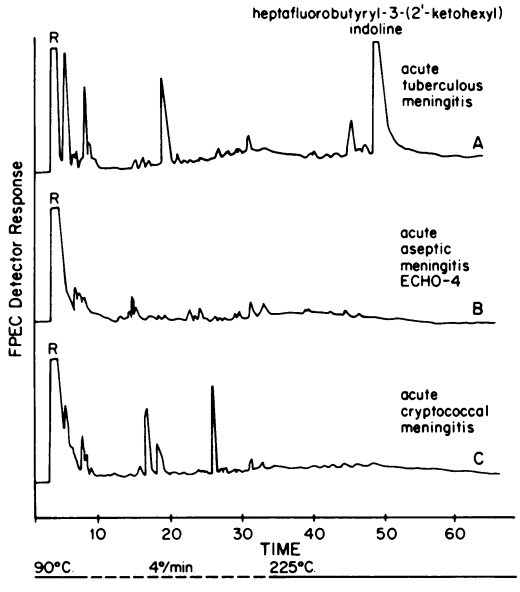


FIG. 1. *FPEC GLC chromatograms of HFBA derivatives prepared from pH 10 chloroform extractions. Analyses were made on a 3% OV-1 column.*

RESULTS AND DISCUSSION

The computerized mass spectrum of heptafluorobutyryl-3-(2'-ketohexyl)indoline with background correction is shown in Fig. 2. Most of the fragment ions observed in the spectrum were produced by a multicenter cleavage of the parent molecule and rearrangement. A strong molecular ion is present at *m/e* 413. A rather intense peak was sometimes observed at *m/e* 412 and a smaller peak at *m/e* 413. This change in the molecular ion position was probably due to pressure depression in the mass spectrometry, which is known to affect aminic compounds in a similar manner. Other intense fragments are present at *m/e* 56, 100, 147, and 216. Of these four fragments, the strongest is found at *m/e* 56, which is the base peak. The next-strongest fragment is found at *m/e* 216. Prominent fragments shown in Fig. 2 were detected by both instruments with electron impact.

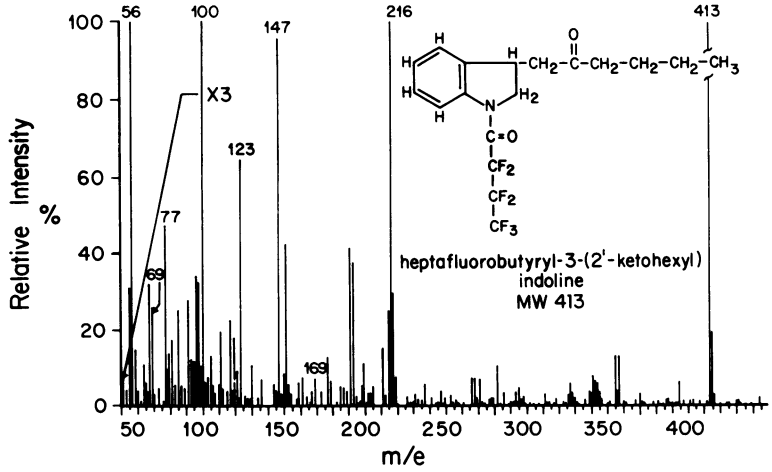
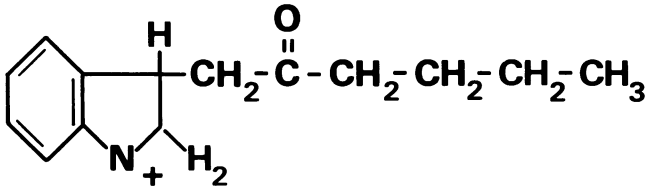
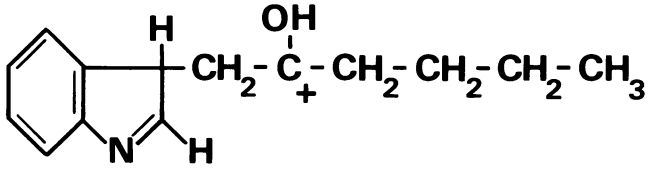


FIG. 2. *Computerized mass spectra of 3-(2'-ketohexyl)indoline with the background component removed by the computer.*

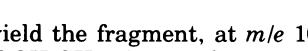
The ion at *m/e* 56 was probably produced by the α cleavage from the parent molecule and a hydrogen rearrangement to produce the fragment $\cdot CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2^+$. The second-strongest fragment (at *m/e* 216) appeared to be produced by a loss of a heptafluorobutyryl group from the molecular ion to give the fragment



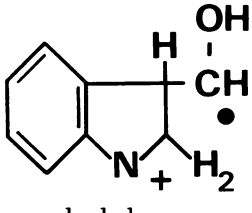
which underwent rearrangement to produce



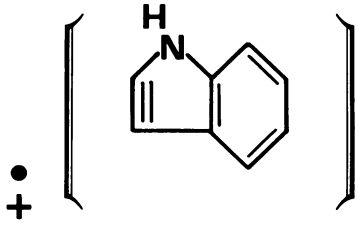
and then further cleavage of the side chain



could yield the fragment, at *m/e* 100, $CH_2\text{-}C^+\text{-}CH_2CH_2CH_2CH_3$. An ion fragment at *m/e* 100 was also produced as a result of the fragmentation of the heptafluorobutyryl group to yield $\dot{C}F_2\text{-}CF_2^+$; therefore, the fragment at *m/e* 100 is probably a combination of both types of cleavage. The moderately intense fragment at *m/e* 147 can logically be depicted as an odd electron ion



resulting from a dual cleavage and rearrangement. The fragment at *m/e* 123 resulted when the parent molecule was stripped of both the heptafluorobutyryl group and the 2'-ketohexyl group to leave



There were other less intense ions of interest at *m/e* 77 indicative of the phenyl ion and fragments at *m/e* 69, 119, and 169 that resulted from the fragmentation of HFBA.

We were not able to obtain 3-(2'-ketohexyl)indoline to compare spectra and retention time; however, chemical ionization studies were performed on the compound, and these studies confirmed the molecular weight as 413. Positive identification would require use of additional chemical techniques such as nuclear magnetic resonance or synthesis of the compound and verification by FPEC GLC and mass spectrometry. It would be almost impossible to obtain enough of the compound from tuberculosis CSF to do nuclear magnetic resonance analysis; therefore, positive identification must await synthesis of the compound. In addition, mass spectral analyses of CSF obtained from different patients were repeated, and similar spectra were obtained from each analysis. We have extracted and derivatized other indole amines to obtain an idea of their mass spectral patterns, solubility characteristics, and behavior in our chromatographic system. The detection limits of 3-(2'-ketohexyl)indoline were not determined, but melatonin, tryptamine, and 5-hydroxytryptamine were extracted and detected, under normal operating conditions with temperature programming, in low nanomole quantities.

The HFBA derivative of melatonin eluted about 6 min ahead of 3-(2'-ketohexyl)indoline when programmed from 90°C, and we used this compound for further studies using isothermal temperature and decreased attenuation of the detector. Under these conditions we were able to reduce the time of analysis for melatonin from 41.6 to 14.8 min and to increase its detection limits to low picomolar range. Although

melatonin and 3-(2′-ketohexyl)indoline possess some different chemical characteristics, without a doubt 3-(2′-ketohexyl)indoline could also be detected faster and in smaller quantity with the isothermal approach. However, other FPEC GLC meningitides pattern characteristics would be lost.

To our knowledge this is the first reported finding of an indoline type of amine appearing in the CSF of patients with acute meningitides. The major indole found in the central nervous system is serotonin. Other indoles (5-methoxytryptamine, *N*-acetylserotonin, and melatonin) have been reported to be localized in the pineal gland (2). The finding that 3-(2′-ketohexyl)-indoline is in the CSF of patients with tuberculous meningitis leaves two prominent questions to be answered: (i) what effect does 3-(2′-ketohexyl)indoline have on the host; and (ii) where does it come from? The origin of 3-(2′-ketohexyl)indoline is unknown, but it could originate as a host response to the infectious agent, a metabolite of the organism, inflammatory cells, or diseased tissue. It is possible that it could be a component extracted from the cell wall of the infectious agent or cellular debris. The last possibility seems unlikely since centrifugation of the sample prior to extraction does not remove the compound. FPEC GLC with its selectivity and sensitivity offers attractive possibilities both for further studies of 3-(2′-ketohexyl)indoline and as a marker for rapid differentiation between some forms of meningitis.

ACKNOWLEDGMENT

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LITERATURE CITED

1. **Brooks, J. B., C. C. Alley, and J. A. Liddle.** 1974. Simultaneous esterification of carboxyl and hydroxyl groups with alcohols and heptafluorobutyric anhydride for analysis by gas chromatography. Anal. Chem. 46:1930–1934.
2. **Koslow, S. H., and A. R. Green.** 1973. Analysis of pineal and brain indole alkylamines by gas chromatography-mass spectrometry. Adv. Biochem. Psychopharmacol. 7:33–43.