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Identification of Phytosterols in Red Oil Extract of Cannabis

Red oil extract of cannabis traditionally is obtained from the ethanol extractable constituents of hemp.[3,4] That portion of the ethanol-extracted material which remains after steam distillation and aqueous washing is distilled under vacuum and constitutes crude red oil, an item of illegal commerce.

White crystalline material was found in red oil extract of Yugoslavian cannabis which had been brought to the U.S. Customs Laboratory for other investigations.[5] In the experience of this laboratory, the occurrence of crystalline material in red oil extract was unusual. Therefore, the crystals were collected, dried under vacuum, and analyzed by gas chromatography and mass spectrometry.

Three principle components were separated by gas chromatography (Fig. 1). Mass spectra obtained on a combined gas chromatograph-mass spectrometer confirmed molecular ions of mass 400 for component A, 412 for component B, and 414 for component C. A high resolution mass spectrum was obtained of the mixture. The exact masses observed for the three molecular ions are compared in Table 1 with the theoretical masses of a series of hydrocarbons containing one oxygen.

TABLE 1—*Molecular ions of cannabis material.*

Observed	Calculated	Formula
400.3726	400.3705	$C_{28}H_{48}O$
412.3673	412.3705	$C_{29}H_{48}O$
414.3860	414.3861	$C_{29}H_{50}O$

Authentic samples of campesterol, stigmasterol, and β-sitosterol were found to have gas chromatographic retention times on two different columns, which were identical with those of components A, B, and C, respectively (Fig. 2).

Mass spectra of the three unknown compounds and the three standard sterols are shown in Figs. 3 through 8. The prominent molecular ions, each accompanied by loss of a methyl radical (15 mass units) and water (18 mass units), are typical of sterols. Loss of the side

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[4] Lerner, M. and Zeffert, J. T. in *Bulletin on Narcotics*, BNUNA, Vol. 20, 1968, pp. 53–54.

[5] Sernec-Avsic, T., *Journal of the Slovene Pharmaceutical Society*, 1970, pp. 27–39. We wish to thank Dr. Sernec-Avsic and the Yugoslavian government for this extract.

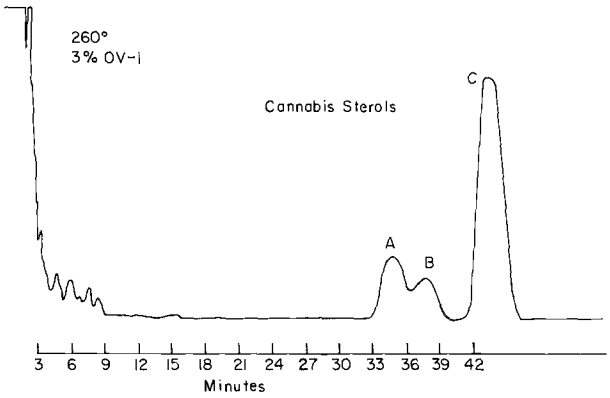

FIG. 1—*Gas chromatogram of cannabis sterols.*

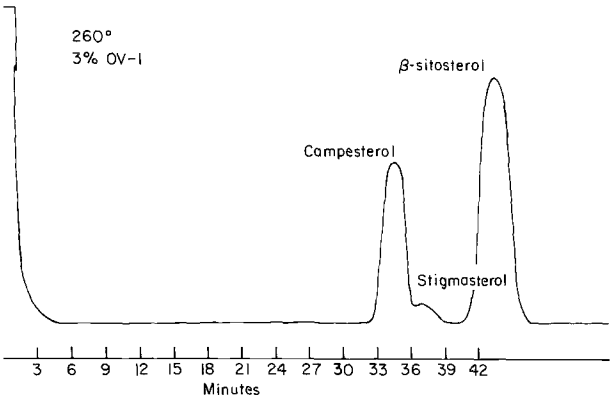

FIG. 2—*Gas chromatogram of campesterol, stigmasterol, and β-sitosterol.*

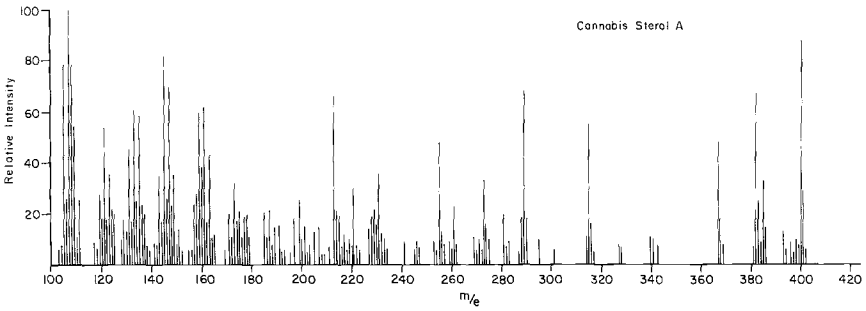

FIG. 3—*Mass spectrum of cannabis sterol A.*

chain leads to ions represented by the peaks at m/e 273 in all the spectra. The additional loss of C_3H_7 (43 mass units) from the molecular ions of stigmasterol and unknown B (m/e 369), and the loss of C_3H_7 accompanied by loss of water (m/e 351) apparently reflect the position of the double bond at C-22, 23, which facilitates cleavage of the allylic isopropyl group.

In view of the coincidence of their gas chromatographic retention times, the similarity of their mass spectra, and their elemental composition ascertained by high resolution mass

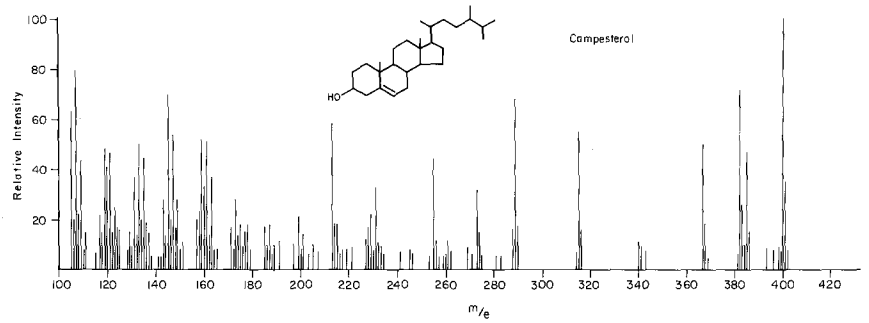

FIG. 4—*Mass spectrum of campesterol.*

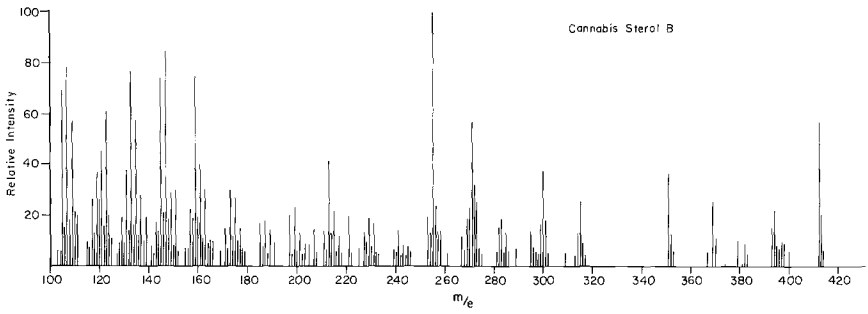

FIG. 5—*Mass spectrum of cannabis sterol B.*

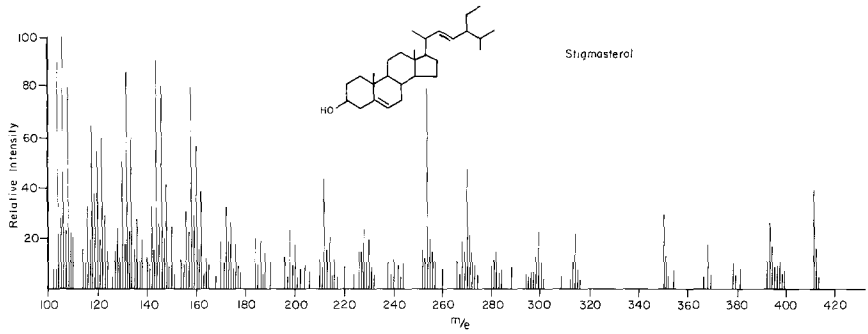

FIG. 6—*Mass spectrum of stigmasterol.*

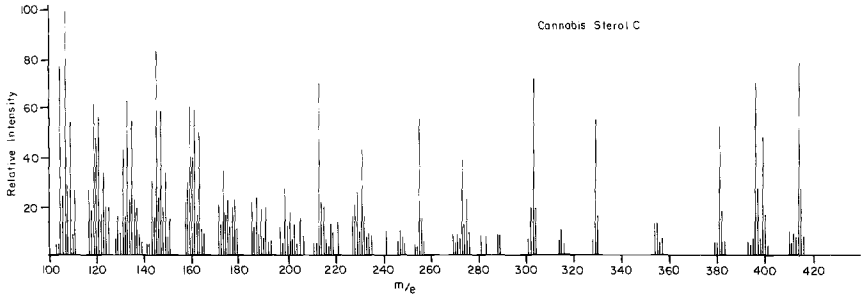

FIG. 7—*Mass spectrum of cannabis sterol C.*

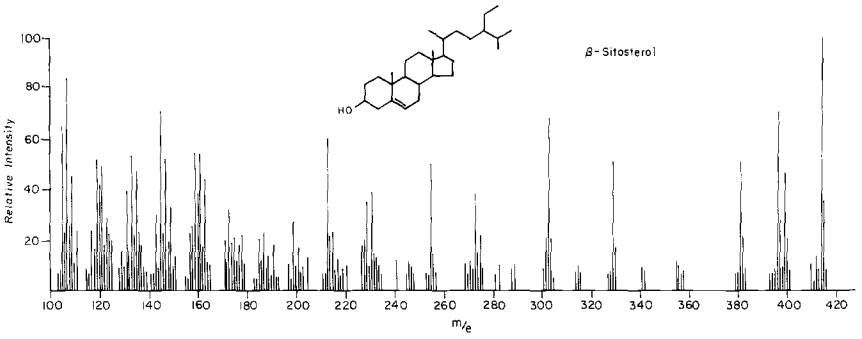

FIG. 8—*Mass spectrum of β-sitosterol.*

spectrometry, unknown A is identified as campesterol, B as stigmasterol, and C as β-sitosterol.

Experimental Procedure

Gas chromatography was carried out on a Barber Colman Model 6000 instrument at 260 C using 6-ft glass columns packed with 3 percent OV-1, and on an LKB combined gas chromatograph-mass spectrometer using a 6-ft glass column packed with 1 percent OV-17, with the temperature programmed to rise from 190 to 270 C at 6 C/min. The spectra in Figs. 3 through 8 were obtained on this instrument at 70 eV, with the separator at 280 C and the source at 290 C. Some variation in peak intensities results from the change in sample pressure across the gas chromatographic peak. The high resolution spectrum was recorded on a photoplate in a CEC 21-110 double focusing mass spectrometer using the direct probe inlet system with the source at 200 C. Authentic samples of campesterol, stigmasterol, and β-sitosterol were obtained from Fluka AG.

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