A more detailed account of this work will be published.

(1) D. C. Monkhouse, L. van Campen, and A. J. Aguiar, *J. Pharm. Sci.*, **62**, 576(1973).

(2) T. O. Oesterling, W. Morozowich, and T. J. Roseman, *ibid.*, **61**, 1861(1972).

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# Constituents of *Cannabis sativa* L. VI: Propyl Homologs in Samples of Known Geographical Origin

**Keyphrases** □ *Cannabis sativa* L.—propyl homologs in samples of known geographic origin □ Cannabidivarin and (−)-Δ⁹-*trans*-tetrahydrocannabivarin—identified in cannabis samples of known geographical origin

*Sir:*

Recent developments in identifying propyl homologs of cannabinoids prompted this communication. We wish to report the presence of cannabidivarin[1] and (−)-Δ⁹-*trans*-tetrahydrocannabivarin in freshly grown cannabis from known geographical locations. We routinely employed GLC and TLC[2] to identify the $C_3$-homologs. Some samples were identified by combined GLC–mass spectrometry[3].

Vollner *et al.* (1) identified cannabidivarin (I) from a sample of hashish in 1969. Gill *et al.* (2) later identified (−)-Δ⁹-*trans*-tetrahydrocannabivarin (II) from a sample of tincture of cannabis. Merkus (3) reported the presence of cannabivarin (III) in samples of Nepal hashish. This research group reported the presence of I and II in a sample of freshly grown *Cannabis sativa* L. from Indian seed stock (IN-B) grown in Mississippi (4).

De Zeeuw *et al.* (5) reported that propyl cannabinoids seem to depend on sample origin: samples from countries like India, Nepal, and Pakistan contained significant amounts of propyl cannabinoids, whereas samples from Middle Eastern and Mediterranean countries contained much lower amounts. Our own findings using only those variants from exact geographical locations confirm and extend the previously thought abundance of propyl cannabinoids in freshly grown *C. sativa* L. (Table I).

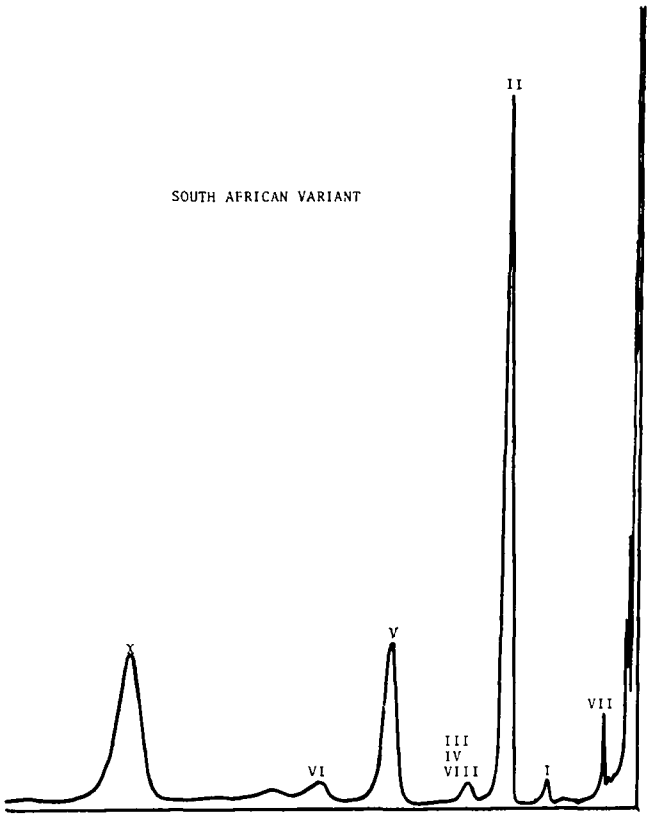


**Figure 1**—*Chromatogram of South African* C. sativa *L. (coded SA-E). Key: I, cannabidivarin; II, (−)-Δ⁹-trans-tetrahydrocannabivarin; III, cannabivarin; IV, cannabichromene; V, (−)-Δ⁹-trans-tetrahydrocannabinol; VI, cannabinol; VII, (−)-Δ⁹-trans-tetrahydrocannabiorcol; VIII, cannabidiol; and X, 4-androstene-3,17-dione (internal standard).*

The percentages of I and II given in Table I are normalized reports. Each cannabinoid is reported as its percentage in regard to total cannabinoid content. These data were obtained by a GLC–computer[4] analysis based on relative retention times of routine cannabis analysis[5].

Figure 1 of an African variant (seed code SA-E) contained 1.70% of I; 53.69% of II; 2.75% of III, cannabichromene (IV), and cannabidiol (VIII); 23.41% of (−)-Δ⁹-*trans*-tetrahydrocannabinol (V); and 4.38% of cannabinol (VI). Peak number VII was tentatively identified as (−)-Δ⁹-*trans*-tetrahydrocannabiorcol[6], first reported by Vree *et al.* (6).

Figure 2 of an Afghanistan variant (seed code AF-A) contained 8.35% of I; 5.34% of II; 12.48% of III, IV, and VIII; 1.94% of cannabigerol monomethyl ether (IX); 58.93% of V; and 2.03% of VI. The age of the material analyzed in Figs. 1 and 2 was 20 weeks. A previous literature report (7) showed that cannabinoid contents vary within each variant according to age.

[1] Since Vollner *et al.* (1) used cannabidivarin (divarinyl group) for the $C_3H_7$ side chain of cannabidiol (olivetyl group $C_5H_{11}$), and Merkus (3) used cannabivarin for the $C_3H_7$ side chain of cannabinol, we shall use the following trivial names: cannabidivarin, (−)-Δ⁹-*trans*-tetrahydrocannabivarin, and cannabivarin. Gill *et al.* (2) used "divarol" for the $C_3H_7$ side chain: (−)-Δ⁹-*trans*-tetrahydrocannabidivarol.

[2] Beckman GC-45, GC-72-5, and GC-5. Procedures described by Turner and Hadley (7) were used. Silica gel G precoated plates from Brinkmann were used for TLC analysis; petroleum ether–ether (4:1) was the solvent.

[3] Varian Series 1400 gas chromatograph interfaced with Dupont 21-492.

[4] Digital PDP-8 computer.

[5] A comprehensive listing of the relative retention times can be found in *Reference 7*.

[6] The methyl side chain of (−)-Δ⁹-*trans*-tetrahydrocannabinol as named by Vree *et al.* (6).

**Table I**—Variants Analyzed for Propyl Homologs

| Geographical Origin | Seed Code | Sex[a] | —Cannabinoids[b]— I | II |
|---|---|---|---|---|
| Afghanistan | AF-A | F | 8.33 | 5.34 |
| | | M | t | + |
| | AF-B | F | + | 7.72 |
| | | M | + | + |
| | AF-C | Y | t | 48.23 |
| Brazil | BZ-A | Y | – | t |
| | | F | – | t |
| Chile | CH-A | M | – | t |
| Canary Islands | CI-A | Y | t | t |
| Czechoslovakia | CZ-A | Y | t | t |
| | | F | t | t |
| | | M | t | t |
| Ethiopia | ET-A | M | t | t |
| France | FR-A | Y | + | t |
| | | F | t | t |
| | | M | t | t |
| | FR-B | Y | t | t |
| | FR-C | F | – | – |
| Ghana | GH-A | X | – | t |
| | GH-B | Y | – | t |
| | | X | – | t |
| | GH-C | Y | – | t |
| | GH-E | F | – | t |
| | | X | – | + |
| India | IN-A | Y | – | – |
| | | F | – | t |
| | | M | – | t |
| | IN-B | Y | + | + |
| | | F | + | 10.63 |
| | | M | + | + |
| | IN-D | Y | – | + |
| | IN-E | F | – | + |
| | | M | – | t |
| | IN-F | Y | – | + |
| | IN-I | Y | + | t |
| Iran | IR-A | F | – | + |
| | | M | – | t |
| Jamaica | JA-A | Y | – | + |
| Japan | JP-A | Y | – | + |
| | JP-B | Y | – | + |
| Korea | KO-A | Y | – | + |
| | | F | – | t |
| | | M | – | t |
| Kenya | KE-A | M | – | t |
| Lebanon | LE-A | F | + | + |
| | | M | – | – |
| Mexico | ME-A | Y | t | t |
| | | F | t | t |
| | | M | t | t |
| Mauritius | MA-A | F | – | + |
| Morocco | MO-A | F | – | – |
| | MO-B | F | – | – |
| | MO-C | F | – | – |
| Manchuria | MN-A | F | – | – |
| Nepal | NE-C | Y | – | + |
| | | F | t | + |
| | | M | t | + |
| Nigeria | NI-D | Y | t | 8.80 |
| Pakistan | PK-A | F | + | + |
| | | M | t | t |
| Peru | PU-A | Y | – | t |
| | | M | – | t |
| Poland | PO-A | Y | – | – |
| | | F | – | – |
| | | M | – | – |
| | PO-B | Y | – | – |
| | | F | – | – |
| | | M | – | – |
| Senegal | SE-A | Y | – | t |
| South Africa | SA-A | Y | t | + |
| | | F | t | 53.69 |
| Sierra Leone | SL-A | F | – | t |
| Sudan | SU-A | F | + | + |
| Thailand | TI-B | Y | – | t |
| | | F | – | t |
| | | M | – | t |
| | TI-C | Y | t | t |
| | | F | t | t |
| | | M | t | t |
| | | Y | t | t |
| | | F | t | t |
| | | M | t | t |
| | TI-D | Y | t | t |
| | | F | t | t |
| | | M | – | t |
| Turkey | TU-A | Y | – | – |
| | | F | – | – |
| | | M | – | – |
| | TU-C | Y | – | – |
| | | F | – | – |
| | | M | – | – |
| USA | US-A | Y | – | t |
| Viet Nam | VN-B | Y | – | t |
| | | F | – | t |
| | | M | – | t |

[a] Y = young plants prior to sexual differentiation, F = female, M = male, and X = mixture of male and female. [b] – = possible (very small peaks), t = trace amounts (approximately 1%), and + = 1–5%.

Therefore, the data in Table I showing variation of I and II in regard to geographical location are not unusual and would be anticipated.

Just as the $C_5H_{11}$ (olivetyl group) side-chain neutral cannabinoids exist predominately as their carboxylic

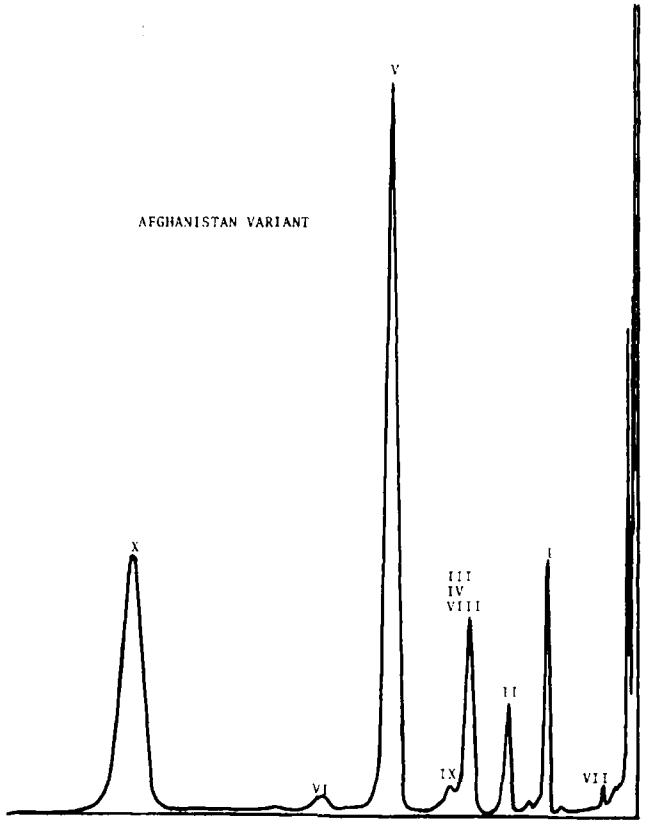


**Figure 2**—*Chromatogram of Afghanistan* C. sativa *L. (coded AF-A). Key: I, cannabidivarin; II, (−)-Δ⁹-trans-tetrahydrocannabivarin; III, cannabivarin; IV, cannabichromene; V, (−)-Δ⁹-trans-tetrahydrocannabinol; VI, cannabinol; VII, (−)-Δ⁹-trans-tetrahydrocannabiorcol; VIII, cannabidiol; IX, cannabigerol monomethyl ether; and X, 4-androstene-3,17-dione (internal standard).*

Table II—Mass Spectral Data

| Trimethylsilyl Derivatives of Cannabinoids | *m/e* |
|---|---|
| (−)-$\Delta^9$-*trans*-Tetrahydrocannabinolic acid | 502 |
| (−)-$\Delta^9$-*trans*-Tetrahydrocannabivarinic acid | 474 |
| Cannabidiolic acid | 574 |
| Cannabidivarinic acid | 546 |

acid derivatives in fresh *C. sativa* L. (8), so do the $C_3H_7$ (divarinyl group) side chains. GLC–mass spectrometry analysis of an Indian (IN-B) variant, silylated according to a reported procedure (9), contained a significant *m/e* at 546, corresponding to the trisilylated (trimethylsilyl ester bisether) derivative of I. Additionally, a significant *m/e* at 474 is indicative of disilylated (trimethylsilyl ester–ether) II. These data agree with the mass spectral data obtained for silylated acid derivatives of VIII and V, having *m/e*'s of 574 and 502, respectively (Table II). TLC was employed for identification of III, which has a relative retention time corresponding to IV and VIII. Compound III was not clearly observed in fresh samples of cannabis but was observed when samples containing II were heated. Thus, no data are presently available on the acid derivative of III.

Propyl homologs are, indeed, found in aerial parts of *C. sativa* L. plant material from many geographical locations. The abundances of propyl homologs vary, as do the pentyl homologs, in regard to geographical origin. However, we do not believe it feasible to determine exact geographic origin using the propyl homologs as "locators." Moreover, the propyl homologs exist predominately as their carboxylic acid derivatives as do the pentyl homologs.

(1) L. Vollner, D. Bieniek, and F. Korte, *Tetrahedron Lett.*, **1969**, 145.
(2) E. W. Gill, W. D. M. Paton, and R. G. Pertwee, *Nature*, **228**, 134(1970).
(3) F. W. H. M. Merkus, *ibid.*, **232**, 579(1971).
(4) P. S. Fetterman and C. E. Turner, *J. Pharm. Sci.*, **61**, 1476 (1972).
(5) R. A. de Zeeuw, J. Wijsbeek, D. D. Breimer, T. B. Vree, C. A. M. van Ginneken, and J. M. van Rossum. *Science*, **175**, 4023 (1972).
(6) T. B. Vree, D. D. Breimer, C. A. M. van Ginneken, J. M. van Rossum, R. A. de Zeeuw, and A. H. White, *Clin. Chim. Acta*, **34**, 365 (1971).
(7) C. E. Turner and K. Hadley, *J. Pharm. Sci.*, **62**, 251(1973).
(8) P. S. Fetterman, N. J. Doorenbos, E. S. Keith, and M. W. Quimby, *Experientia*, **27**, 988(1971).
(9) C. E. Turner and K. Hadley, *J. Pharm. Sci.*, **62**, 1083(1973).

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Voucher specimens are located in the *Cannabis sativa* L. Herbarium, School of Pharmacy, University of Mississippi.

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# Coacervate Formation by Inorganic Salts with Benzalkonium Chloride

**Keyphrases** ☐ Coacervate formation—sodium chloride and sodium carbonate with benzalkonium chloride ☐ Sodium chloride—coacervate formation with benzalkonium chloride ☐ Sodium carbonate—coacervate formation with benzalkonium chloride, effect of concentration on coacervate volume and refractive index ☐ Inorganic salts, sodium chloride and sodium carbonate—coacervate formation with benzalkonium chloride ☐ Benzalkonium chloride—coacervate formation with sodium chloride and sodium carbonate

*Sir:*

A number of dilute aqueous solutions of proteins (1), polyelectrolytes (2), association colloids (3–5), carbonates (6), and lipids (6) have been investigated and found capable of forming coacervates under proper conditions. The essential feature of the coacervation phenomenon is the spontaneous separation of a homogeneous macromolecular or microionic aqueous solution into two immiscible aqueous solution phases. One aqueous layer contains most of the colloid and is termed the coacervate, while the second aqueous layer is colloid poor and is termed the equilibrium liquid. These coacervate systems are of growing general scientific (7, 8) and biological (9) interest.

The term biphasic coacervate system refers to the coacervate phase in contact with its equilibrium liquid phase. The term monophasic solution refers to the one-phase micellar solution.

Coacervation has been observed in some dilute soap solutions, and it has been reported (5) that coacervation in cationic detergent systems shows pronounced specificity to the anion and lesser sensitivity to the cation of the added electrolyte. Shah *et al.* (3) reported coacervate formation between the cationic surfactant, benzalkonium chloride, and the organic aromatic compound, sodium salicylate.

This communication reports on coacervate formation by sodium salts of inorganic monovalent and

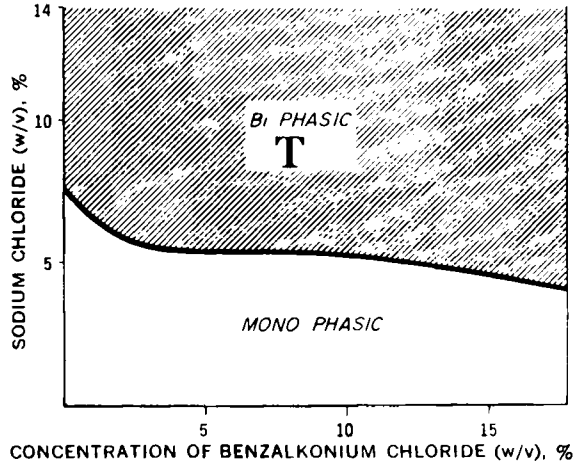


**Figure 1**—*Phase transition diagram of sodium chloride-benzalkonium chloride coacervate system at 25°. Shaded area [T] represents the region in which biphasic coacervate system can be formed with lighter coacervate phase.*