diesters occurring in croton oil. It is interesting to note here that in case of croton oil several esters of 4-deoxy-4α-phorbol have been shown to be present as minor constituents[7]. However, it may be argued[7] that these esters in fact are not the native constituents: they may be formed from naturally occurring (most sensitive[15,18,19]) esters of 4-deoxyphorbol by epimerization under the acidic milieu of croton oil or of the croton seeds themselves. Although the diterpene moiety of 4-deoxy-16-hydroxyphorbol esters, as for example in Croton factors F_2 and F_2', is susceptible towards alcali catalyzed epimerization in 4-position, no corresponding 4α-epimeric esters were isolated from roots of *Croton flavens.*

Croton factors from fruits. A preliminary investigation of the fruits of Croton flavens, each containing three seeds, yielded mixtures of croton factors exhibiting the same R_f values and similar irritant activities as the factors isolated from the roots. The presence in these mixtures of the parent alcohols 16-hydroxyphorbol (**1**) and 4-deoxy-16-hydroxyphorbol (**5**) was demonstrated by transesterification and subsequent thin layer chromatography. The contents (by weight %) of esters in this material as judged from the amounts of **1** and **5** is lower than that of the roots. According to the NMR-spectra of the mixtures of esters, the corresponding croton factors carry an acetate moiety. Further, the presence of long chain acid moieties was demonstrated by transesterification/gas chromatography.

Biological activities of some of the Croton factors described. In our semiquantitative standard assay on the back skin of mice with 7,12-dimethyl-benz(a)anthracene as initiator[7], the methanol extract of the roots and the irritant Croton factors F_1 (16-hydroxyphorbol ester type) and F_2 (4-deoxy-16-hydroxyphorbol ester type) were shown to exhibit cocarcinogenic in terms of tumor promoting activities comparable to that of croton oil factor A_1 (TPA). The corresponding factors of the cryptic irritant type (F_1' and F_2') exhibited little if any cocarcinogenic activity[20]. Because of lack of material, the rest of the croton oil factors isolated was not available for assay of tumor promoting activity.

These findings strongly suggest that the exceptionally high rate of esophageal cancer on Curaçao may be causally related to cocarcinogenic phorbol derivatives ingested by the widespread and frequent use of *Croton flavens* on this island according to local habits[1,2]. Some ideas as to the origin of the initiator(s) involved have been discussed[20]. In addition to a large body of already existing indirect evidence[4,21], the present findings illustrate the role which cocarcinogens of the tumor promoter type may play in the etiology of human cancer as 2nd order carcinogenic risk factors[3,4,21].

*Dedicated to Prof. Dr H.P. Rusch, Director Wisconsin Clinical Cancer Center, Madison, Wis. USA, on occasion of his 70th birthday. We wish to thank Dr J.F. Morton, Morton Collectanea, University of Miami, Florida, USA, for kindly supplying us with the plant material.

1 J.F. Morton, Econ. Bot. *25*, 457 (1971).
2 J.F. Morton, Cancer Res. *28*, 2268 (1968).
3 For definitions of terms see E. Hecker, Int. J. Cancer *18*, 122 (1976); Z. Krebsforsch. *86*, 219 (1976); GANN *67*, 471 (1976); WHO-Bull. *54*, 1 (1976) and Hecker[4].
4 E. Hecker, Symp. Mechanisms of Tumor Promotion and Cocarcinogenesis, March 1977, Gatlinburg, Tennessee, vol. 2, p. 11. Raven Press, New York 1978.
5 R.W. O'Gara, C. Lee and J.F. Morton, J. natl. Cancer Inst. *46*, 1131 (1971).
6 L.J. Dunham, R.H. Sheets and J.F. Morton, J. natl. Cancer Inst. *53*, 1259 (1974).
7 E. Hecker and R. Schmidt, Progr. Chem. Org. Natur. Prod. *31*, 377 (1974); E. Hecker, in: Methods in Cancer Research, vol. VI, p. 439. Ed. H. Busch, Academic Press, 1971.
8 R. Ratcliffe and R. Rodehorst, J. org. Chem. *35*, 4000 (1970).
9 G. Kreibich and E. Hecker, Z. Krebsforsch. *74*, 448 (1970).
10 C. v. Szczepanski, H.U. Schairer, M. Gschwendt and E. Hecker, Liebigs Ann. Chem. *705*, 199 (1967).
11 H.U. Schairer, H.W. Thielmann, M. Gschwendt, G. Kreibich, R. Schmidt und E. Hecker, Z. Naturforsch. *23b*, 1430 (1968).
12 T. Okuda, T. Yoshida, S. Koike and N. Toh, Phytochemistry *14*, 509 (1975).
13 We are gratefully indebted to Dr T. Okuda, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima Okayama, Japan, for the specimen of 12-O-hexadecanoyl-16-hydroxyphorbol-13-acetate.
14 P. Jakobi, E. Härle, H.U. Schairer and E. Hecker, Liebigs Ann. Chem. *741*, 13 (1970); M. Gschwendt, E. Härle and E. Hecker, Z. Naturforsch. *23b*, 1579 (1968).
15 G. Fürstenberger and E. Hecker, Tetrahedron Lett. *1977*, 925.
16 M. Gschwendt and E. Hecker, Z. Naturforsch. *23b*, 1584 (1968).
17 M. Gschwendt and E. Hecker, Z. Krebsforsch. *80*, 335 (1973).
18 G. Fürstenberger and E. Hecker, Experientia *33*, 986 (1977).
19 A. Miana, R. Schmidt, E. Hecker, M. Shamma, J.L. Moniot and M. Kiamuddin, Z. Krebsforsch. *32b*, 727 (1977).
20 E. Hecker and J. Weber, 7th Int. Symp. Biological Characterization of Human Tumors, Budapest, April 1977, Abstracts, p. 52. See also Proceedings, Excerpta Medica, in press, and J. Weber, Ph.D.-thesis, University of Heidelberg (1976).
21 E. Hecker, 10th Symp. Int. Union of Pure and Applied Chemistry (IUPAC) in the Chemistry of Natural Products, August 1976, Dunedin, New Zealand; for full text of the invited lecture, Pure appl. Chem. *49*, 1423 (1977).

Isotrinervi-2β-ol. Structural isomers in the defense secretions of allopatric populations of the termite *Trinervitermes gratiosus*[1]

G.D. Prestwich

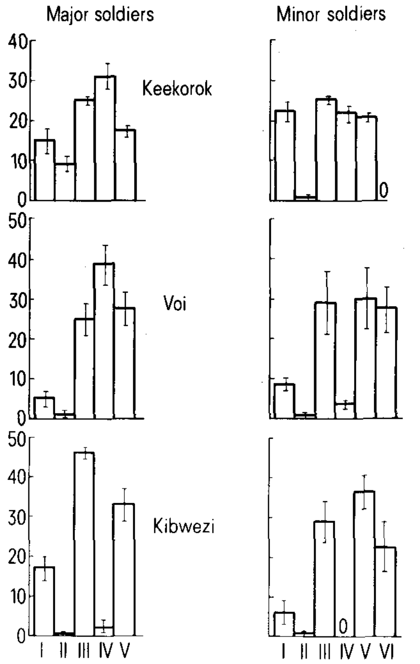
Department of Chemistry, State University of New York, Stony Brook (New York 11794, USA), 19 December 1977

Summary. The defense secretions of 3 allopatric populations of the nasute termite *Trinervitermes gratiosus* were analyzed. One population afforded a new trinervitene, isotrinervi-2β-ol, a missing link in the hypothetical biosynthesis of the trinervitenes. Populations could be readily distinguished on the basis of the chromatographic profiles of their major and minor soldier frontal gland secretions.

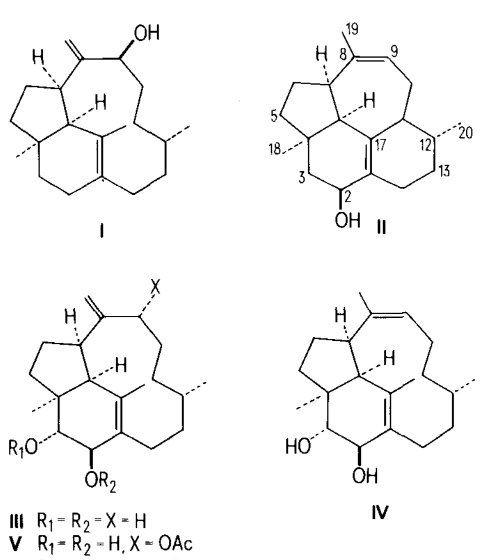
Nasute termite soldiers (Isoptera: Termitidae: Nasutitermitinae) eject an irritating, sticky defense secretion when provoked. Considerable progress has been made in the elucidation of the structures of the individual diterpenoid 'resins'[2], the overall chemical composition of the secretions[3], and the use of the secretions in defense[4]. Interspecific variations in defense secretion chemistry are commonplace among termites[2]. However, no examples of intraspecific variation have been reported. My investigations of 3 allopatric populations of the East African grass-feeding termite *Trinervitermes gratiosus* (Sjöstedt) demonstrate significant differences in the chemical compositions of the soldier secretions within a single species, including the production of isomeric compounds in different populations.

Termite soldiers were collected from mounds along linear transects (20-80 km) of each of the following regions of Kenya: Voi (60 km), Kibwezi (80 km), and Keekorok (20 km). The materials were processed and the mono- and diterpene compositions were analyzed as described previously[3].

A mixture of compounds **I** and **II** was isolated by column chromatography (Florisil, 5% ethyl acetate-benzene) of the hexane extract of 2000 major soldier heads of *T. gratiosus* collected in the Keekorok area. Further purification by liquid chromatography (1×50 cm Lichrosorb SI60, 30 μm, 4.0 ml/min of 20% ether-hexane) afforded 25 mg of pure **II**. The electron-impact mass spectrum[5] (70 eV) of **II** showed high-mass fragments at m/e 288 (5%, M^+) and 270 (1%, M^+-H_2O), and a base peak at m/e 41. The chemical ionization mass spectra (methane or isobutane) showed small $M^+ + 1$ peaks at m/e 289 and a base peak at 271 ($M^+ + 1$ - H_2O). The IR-spectrum (10% in $CHCl_3$) showed hydroxyl stretching bands at 3640 (sharp, free OH) and 3450 cm^{-1} (weak, broad, H-bonded). The ^{1}H-NMR[6] showed the following important resonances: δ5.11 (ddq, 12, 6, 1 Hz, 1H, H-9), 3.92 (dd, 9.6, 7 Hz, 1H, H-2), 1.64 (s, 3H, H-17), 1.58 (s, 3H, H-19), 0.92 (s, 3H, H-18) and 0.81 (d, 6.4 Hz, H-20). The ^{13}C-NMR showed olefinic carbon resonances at 131.1, 127.3, 127.2 (tetrasubstituted) and 127.5 (trisubstituted, C-9), and a single carbinol carbon at 72.2 (C-2). Other carbon resonances (except C-3) were also in close agreement with the corresponding values for the previously-studied[2b] isotrinervi-$2\beta,3\alpha$-diol (TG-3, **IV**).



Trinervitene distribution in the frontal gland secretions of *Trinervitermes gratiosus* soldiers from 3 locations. The horizontal axes indicate order of GLC elution (3% OV-17, 2 mm×3 m, 200–250°, 5°/min). Structures are given in the scheme. The vertical scales indicate mean relative percents of each components as determined from the integrated GLC traces. Sample sizes were: Keekorok, n=6; Voi, n=13, Kibwezi, n=7. Error bars are $\pm 1\sigma$.



III $R_1 = R_2 = X = H$
V $R_1 = R_2 = H, X = OAc$
VI $R_1 = R_2 = Ac, X = OH$

Scheme. Principal diterpenoid constituents of *Trinervitermes gratiosus* soldier frontal gland secretions.

Addition of the shift reagent Eu(fod)$_3$ resulted in the following new resonance positions (negative values in parentheses represent ppm shifts downfield at L/S=0.33): δ6.71 (t, 8 Hz, H-2, −2.8), 5.37 (m, H-9, −0.2), 3.85 (br d, 8 Hz, H-3, about −2), 2.41 (s, H-18, −1.5), 2.02 (br s, H-17, −0.4), 1.77 (d, 1.2 Hz, H-19, −0.2), 0.97 (d, 6.4 Hz, H-20, −0.15), 0.77 (br s, H-5 and H-13, about +1.5). Selective decoupling experiments confirmed these assignments. Of particular interest is the large upfield shift of the methylenes in the 'shielding cone' (O-Eu-H angle between 55 and 125°) of the paramagnetic species[7]. This is consistent with an axial nature for the 2β-OH and its concomitant sharp free O-H stretch, since models suggest that a substituent in this position must lie in the concave, sterically congested area of the dome-like trinervitene molecule[2,a and b]. Trinervitenes possessing the exocyclic 8(19) double bond have the 6-membered ring in a boat-like conformation placing the 2β and 3α substituents in equatorial positions[2,a and b]. However, isomerization to the endocyclic 8(9) double bond necessitates a half-chair for the 6-membered ring due to the reduced flexibility of the bridging 11-membered ring.

Gas chromatographic analysis (5% Carbowax 20 M, 4 mm×2 m, 60 °C) of the monoterpenes produced 2 surprising results. Major soldiers of all 3 populations produced α-pinene almost exclusively ($\geq 97\%$). Minor soldiers, however, produced mixtures of α-pinene, β-pinene, camphene, and limonene in the Voi and Kibwezi populations, but only α-pinene in the Keekorok group. Furthermore, the terpene alcohols (m/e 156) reported[3] for the former 2 populations were totally absent from both major and minor soldiers of the Keekorok termites.

The data comparing the distribution of the diterpenoid constituents of the soldier secretions are summarized in the figure. Analysis of individual soldiers by GLC and TLC[3] demonstrated homogeneity within a mound; analysis of 10–20 soldiers per mound showed homogeneity in a given population. No intermediate secretion types were found; in fact, in areas between populations, no termite mounds of this species were observed.

3 dramatic differences are apparent from these data. 1. Minor soldiers in the Keekorok population produce no TG-4 (**VI**), a major constituent in the other 2 populations. 2. Major soldiers from Kibwezi are depleted in both isotrinervitenes **II** and **IV**. 3. Only the Keekorok termites produce appreciable amounts of the new isotrinervi-2β-ol (**II**).

Petri-dish colonies of *T. gratiosus* were laboratory-reared in Nairobi using field-collected Voi-type alates. They were fed dried grass from an area not inhabited by *T. gratiosus*. After 6 months, 4 surviving incipient colonies were sacrificed, yielding about 10 minor soldiers each. GLC analysis of the secretions showed a trinervitene distribution characteristic of the field-collected Voi minor soldiers. These preliminary data suggest genetic patterning of the enzymes responsible for trinervitene biosynthesis. Furthermore, the discovery of the new trinervitene **II** lends support to a hypothetical biosynthesis from intramolecular cyclization of cembrene-like precursors followed by specific hydroxylation at C-2, C-3, C-9, C-13,[2,e and f] or C-17[2,b].

[1] These studies were initiated (1975-76) with partial financial support by the International Centre of Insect Physiology and Ecology, Nairobi, Kenya and NIH Postdoctoral Fellowship AI-05076. Support by the Department of Chemistry of the State University of New York at Stony Brook and the technical assistance of Mr J. Engstrom allowed completion of this investigation.

[2] *a* G.D. Prestwich, S.P. Tanis, J.P. Springer and J. Clardy, J. Am. chem. Soc. *98*, 6061 (1976); *b* G.D. Prestwich, S.P. Tanis F. Pilkiewicz, I. Miura and K. Nakanishi, J. Am. chem. Soc. *98*, 6062 (1976); *c* G.D. Prestwich, B.A. Solheim, J. Clardy, F.G. Pilkiewicz, I. Miura, S.P. Tanis and K. Nakanishi, J. Am. chem. Soc. *99*, 8082 (1977); *d* J. Vrkoč, K. Ubik, L. Dolejš and I. Hrdý, Acta ent. bohemoslov. *70*, 74 (1973); *e* J. Vrkoč, M. Buděšínský, J. Křeček and I. Hrdý, Proc. Int. Congr. IUSSI, *8*, 320 (1977); *f* J. Vrkoč, M. Buděšínský, and P. Sedmera, Colln. Czech. chem. Commun, in press (1978).

[3] G.D. Prestwich, Insect Biochem. *7*, 91 (1977).

[4] *a* T. Eisner, I. Kriston and D. Aneshansley, Behav. Ecol. Sociobiol. *1*, 83 (1976); *b* W.L. Nutting, M.S. Blum and H.M. Fales, Psyche *81*, 167 (1974); *c* J. Vrkoč, J. Křeček and I. Hrdý, Acta ent. bohemoslov., in press (1978); *d* I. Hrdý, J. Křeček and J. Vrkoč, Proc. Int. Congr. IUSSI, *8*, 303 (1977).

[5] I thank Mr P. Chang and Dr. C. Iden (Stony Brook) for the mass-spectra of compounds I and **II**.

[6] Fourier transform NMR-spectra were obtained in deuteriochloroform solutions on Varian CFT-20 spectrometers operating at 80 MHz for ^1H and 20 MHz for ^{13}C. Data are given in ppm downfield from $(CH_3)_4Si = 0$.

[7] B.L. Shapiro, J.R. Hlubuček, G.R. Sullivan and L.F. Johnson, J. Am. chem. Soc. *93*, 3281 (1971).

The scent scale substances of male *Pieris* butterflies *(Pieris melete* and *Pieris napi)*

N. Hayashi, Y. Kuwahara[1,2] and H. Komae

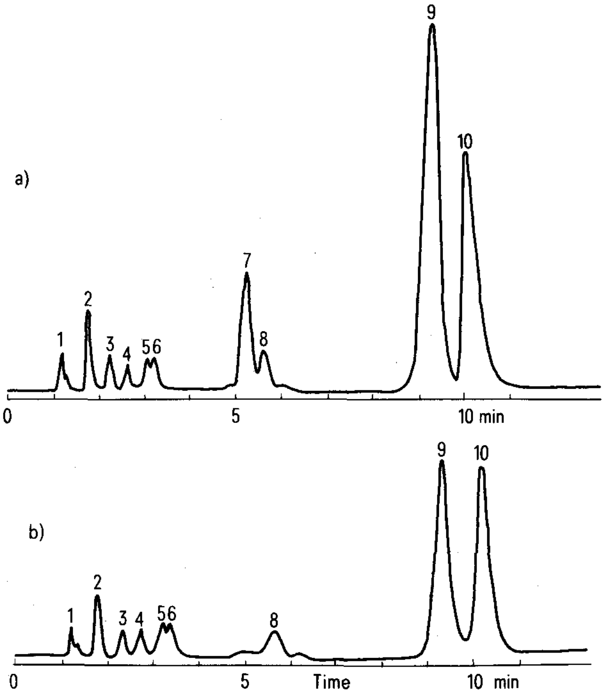
Study of Environmental Science, Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730 (Japan),

27 October 1977

Summary. 7 monoterpenes, *α*-pinene, *β*-pinene, myrcene, p-cymene, limonene, neral and geranial, were identified together with n-undecane in the scent scales of *P. melete*, and also, together with considerable amounts of linalool, in the scent scales of *P. napi*. Content ratio of neral to geranial in *P. melete* ranged from 0.77 to 1.04, and that in *P. napi* from 1.84 to 2.43. The present chemotaxonomic approach shows the 2 species of *Pieris* to be distinct.

Some studies on the chemical structure and physiological functions of scent scale substances of male butterflies have been reported: e.g. the presence of neral, geranial and geraniol in the scent scales of *P. napi* found by Bergström et al.[3], and the presence of nonan-1-al, hexadecan-1-ol, hexadecan-1-ol acetate and muurol (or amorphol) in the scent scales of *Lycalides argyrognomone* observed by Lundgren et al.[4]. It was said that these substances function as an aphrodisiac[3], like the hair pencil secretions of Danoid butterflies[5-8]. The scent scale substances[9] of 2 species (*Pieris melete* Ménétriès and *Pieris napi japonica* Shirôzu) of Pieridae in Japan are investigated in this paper.

Material and method. The butterflies were collected in the mountainous district in Hiroshima Prefecture. The wings of 3 male adults were extracted with n-pentane (5 ml) at room temperature. The extracts were concentrated to 10 µl under nitrogen stream at room temperature. 2 µl of each sample were injected into gas chromatograph-mass spectrometer. The JGC-20KP gas chromatograph equipped with columns was coupled with the JMS-D 100 mass spectrometer (JEOL Co. Ltd., Japan) and was operated under the following conditions: column, 3% OV-1 gas-chrom Q (100-200 mesh) glass column (1 m×3 mm); column temperature programmed 50-150 °C (5 °C/min); injection temperature 200 °C; ionizing voltage 20 and 70 eV. From each of the mass spectra determined, the mass units and intensity of 3 abundant ions, base (H_1), 2-highest (H_2), and 3-highest (H_3) ions, together with molecular ion (M^+), were obtained (table). Based on agreement of these spectral patterns with authentic ones, identification was carried out. For quantitative analysis of geranial and neral, the Hitachi 063 type gas chromatograph equipped with flame ionization detector was used. The gas chromatograph was operated at 120 °C, using PEGS (polyethylene glycol succinate on chromosorb WAW (60-80 mesh) 15%) column (2 m×3 mm). The relative percentage of individual constituents were determined by integration and summation of peak area with electric digital integrator. The individual variation of the content ratio of neral to geranial was obtained from 12 heads of *P. melete* and 9 heads of *P. napi*.



Gas chromatogram of the scent scale substances of *P. napi* (a) and *P. melete* (b). 1. Unknown, 2. *α*-pinene, 3. *β*-pinene, 4. myrcene, 5. p-cymene, 6. limonene, 7. linalool, 8. n-undecane, 9. neral, 10. geranial.