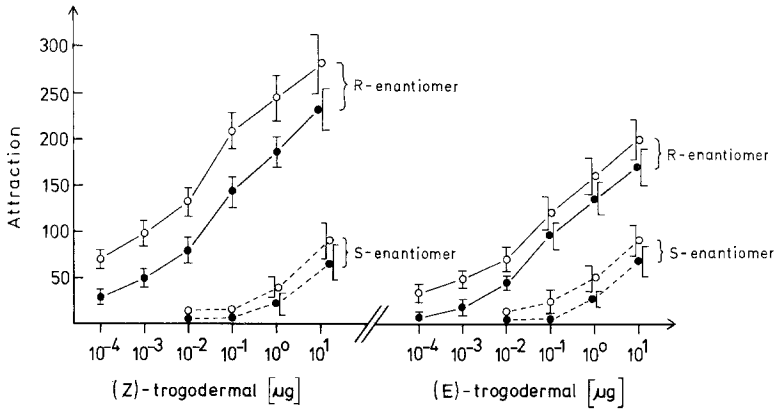

Fig. 2. Attraction of unmated male khapra beetles of two strains to graded levels of the R-(−)- and S-(+)-enantiomers of (*Z*)- and (*E*)-trogodermal in an olfactometer arena [4]. Attraction is expressed as the (average number of males attracted to an enantiomer/average number of males attracted by one female) × 100. Each point represents the average response of 60 male *T. granarium* (4–6 days after eclosion) and the relevant standard deviation (vertical line). o——o, o---o Firenze strain; ●——●, ●---● Seewiesen strain

Table 1. Copulation of unmated male *Trogoderma granarium* induced by different enantiomers of trogodermal

	Threshold level of copulation [µg]			
Enantiomers of trogodermal	R-(−)-*Z*	R-(−)-*E*	S-(+)-*Z*	S-(+)-*E*
Firenze strain	10^{-5}	10^{-3}	10^{0}	10^{0}
Seewiesen strain	10^{-4}	10^{-3}	10^{0}	10^{0}

tiveness and copulation, while the corresponding S-(+)-enantiomers exert a relatively low responsiveness in males of two different strains of the khapra beetle.

Rossi et al. [9] have speculated that the reproductive isolation between *T. granarium* and *T. inclusum* (=*T. versicolor*) is partly due to the release of different enantiomers of trogodermal. However this assumption can hardly be correct, as the males of *T. granarium* and *T. inclusum* are cross-attracted to the females of either species [2, 15], probably due to emission of R-(−)-(*Z*)-trogodermal by both, and since the reproductive isolation between them depends on sexual incompatibility [16, 17].

Our results are in sharp contrast with previous reports by Rossi et al. [7–9], ascribing a high degree of attractiveness for male *T. granarium* of the Firenze strain to the S-(+)-enantiomers, but not to the R-(−)-enantiomers, of (*Z*)- and (*E*)-trogodermal. Considering that the same strain and bioassay [4] were used throughout [7–9], the above contradiction is even more striking. However, our results are in complete agreement with those of Silverstein et al. [13] and Levinson and Mori [10] describing a high degree of attraction for male *T. granarium*, *T. inclusum* and *T. variabile* to R-(−)-(*Z*)-trogodermal and for male *T. granarium* and *T. glabrum* to R-(−)-(*E*)-trogodermal, but a 10^2 to 10^3 fold lower extent of attraction to the corresponding S-(+)-enantiomers. There is no fundamental difference between the Seewiesen and Firenze strains of *Trogoderma granarium* concerning olfactory and mating behaviour of males induced by the R-(−)- and the S-(+)-enantiomers of (Z)- and (*E*)-trogodermal.

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1. Levinson, H.Z., Bar Ilan, A.R.: Riv. Parasitol. *28*, 27 (1967)
2. Levinson, H.Z., Bar Ilan, A.R.: J. Insect Physiol. *16*, 561 (1970)
3. Levinson, H.Z., Levinson, A.R.: Naturwissenschaften *60*, 352 (1973)
4. Levinson, A.R., et al.: J. Chem. Ecol. *4*, 95 (1978)
5. Cross, J.H., et al.: ibid. *2*, 457 (1976)
6. Greenblatt, R.E., et al.: ibid. *3*, 337 (1977)
7. Rossi, R., Carpita, A.: Tetrahedron *33*, 2447 (1977)
8. Rossi, R., Niccoli, A.: Naturwissenschaften *65*, 259 (1978)
9. Rossi, R., et al.: Tetrahedron *35*, 2039 (1979); Naturwissenschaften *66*, 211 (1979)
10. Mori, K., et al.: Tetrahedron *34*, 3119 (1978)
11. Suguro, T., Mori, K.: Agric. Biol. Chem. *43*, 409 (1979)
12. Levinson, H.Z., Mori, K.: Naturwissenschaften *67*, 148 (1980)
13. Silverstein, R.M., et al.: J. Chem. Ecol. *6*, 911 (1980)
14. Levinson, H.Z.: Naturwissenschaften *62*, 272 (1975)
15. Vick, K.W., et al.: Ann. Entomol. Soc. Am. *63*, 379 (1970)
16. Beal, R.S., Spittler, G.H.: Proc. Ent. Soc. Wash. *61*, 1 (1959)
17. Strong, R.G., Arndt, R.G.: J. Econ. Ent. *55*, 445 (1962)

Formation of Podophyllotoxins by *Podophyllum peltatum* Tissue Cultures

P.G. Kadkade

GTE Laboratories, Inc., Waltham, Massachusetts, USA

Podophyllum peltatum, a herb which is common in the whole deciduous forest region of eastern North America [1]. Rhizomes of *P. peltatum* is known to contain lignanes of which podophyllotoxins are used against certain virus and skin cancer diseases [2]. Despite its high medicinal value, plants are still collected in the wild [3]. Harvesting from the wild is a laborious job and the commercial production of podophyllotoxins will ultimately depend on the easy and cheap availability of the raw materials. This communication describes some preliminary studies on the accumula-

tion of podophyllotoxins in callus cultures derived from *P. peltatum*.

Undifferentiated callus tissue was initiated from sterile rhizome segments and grown statically at 25±1 °C with 16 h daily illumination (Gro-Lux lamps, 360 μW/cm²), on a modified Murashige and Skoog's medium [4] supplemented with kinetin (0.2 mg/l), 2,4-dichlorophenoxyacetic acid (2,4-D, 1 mg/l) and casmino acid (500 mg/l) solidified with agar (0.5%). Eight-week-old callus tissues were dried, ground, extracted continuously with hot 90% ethanol and the combined ethanol extracts were concentrated under reduced pressure. The concentrate was suspended in water and the mixture was shaken with chloroform. The chloroform-soluble fractions were combined, evaporated under vacuum and partitioned between hexane and 20% methanol (1:1). The methanolic extract was concentrated in vacuo, chromatographed on silica gel 60 packed in benzene and eluted with benzene, benzene-chloroform (2:1), chloroform and chloroform-methanol [5]. The fraction eluted with chloroform along with the standard reference podophyllotoxins was analyzed by TLC on air-dried silica gel G (wet thickness 250 μm) plates, developed with chloroform-methanol (92:8) as the solvent system. The developed plates were air-dried and visualized under UV light. The corresponding compounds in the fraction separated by preparative TLC (silica gel G, 500 μm thickness) were marked, scraped from developed unsprayed plates, eluted with the same solvent system and rechromatographed by TLC to ascertain the purity of the compounds and repeatedly crystallized from methanol. The first polar band resolved a small amount of a mixture in which the presence of α-peltatin was revealed on the chromatoplates. The second polar band yielded podophyllotoxin and β-peltatin. Authenticity of the isolated podophyllotoxins was substantiated by TLC, mp, UV and IR spectral studies and compared with the data reported for standard podophyllotoxins [6].

Podophyllotoxin production in callus tissue decreased strikingly as the concentration of 2,4-D in the medium increased, or kinetin was deficient in the medium. The similar relationship has been observed with callus tissues derived from other plant species [7]. The rhizome callus tissue showed a steady increase in growth index with a maximum up to 8 weeks and then decreased by the tenth week. Podophyllotoxin production (0.64% as determined by gravimetric means) paralleled growth up to the eighth week and then decreased. It is evident from the foregoing that undifferentiated callus tissues of *P. peltatum* are capable of synthesizing podophyllotoxins and could be useful for in vitro production of podophyllotoxins. These findings further suggest that if the synthetic and accumulating powers of these cultures on solid medium can be enhanced and stabilized in suspension cultures an alternative commercial source of podophyllotoxins may have been discovered.

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1. Meijer, W.: Ec. Bot. *28*, 68 (1974)
2. Claus, E.P., et al.: Pharmacognosy 272 (1970)
3. Krochmal, A.: Ec. Bot. *22*, 332 (1968)
4. Murashige, T., Skoog, F.: Physiol. Plant. *15*, 473 (1962)
5. Hokanson, G.C.: Lloydia *41*, 497 (1979)
6. Hartwell, J.L., Schrecker, A.W.: Prog. Chem. Org. Nat. Prod. *15*, 83 (1958); Kupchan, S.M., et al.: J. Pharm. Sci *54*, 659 (1965); Stahl, E.: Thin Layer Chromatography, p. 87, 710. New York: Springer 1969
7. Shiio, I., Ohta, S.: Agric. Biol. Chem. *37*, 1857 (1973); Ohta, S., Yatazawa, M.: ibid. *43*, 2297 (1979); Kadkade, P.G.: Plant Physiol. *65*, 99 (1980)

"Activity Triangles" in Crystal Structures of Cholinergic Agonists

A. Gieren and M. Kokkinidis

Max-Planck-Institut für Biochemie, Abteilung für Strukturforschung I, D-8033 Martinsried/München

On the basis of eight X-ray crystal structure analyses [1, 4] we have derived a structure-activity relationship for cholinergic agonists [2–4] and correlated our results with crystal structures of related compounds found in the literature.

One common feature of the crystal structures of all cholinergic compounds containing the quaternary trimethylammonio methyl group $[(CH_3)_3N^+-CH_2-]$ is a stereospecific orientation of the anions relative to this group. The faces of the tetrahedron formed by this quaternary ammonium group are stereochemically not equivalent, but form three distinct face types (A, B, C; Fig. 1). Face type B appears doubled due to a local mirror plane. We found that the quaternary ammonium group is coordinated by the anions – normally fourfold – in such a way that the N^+-anion vectors intersect the centre of a tetrahedral face perpendicularly and are collinear with the *trans* positioned CH_3- or CH_2-group, respectively. In the ideal case, which is mainly fulfilled for face types A and B, the angle δ (Fig. 1) is ca. 180°.

A detailed comparison of the geometrical parameters and their correlation with the pharmacological behaviour indicates that the anions occupying face type B are expected to have the same orientation relative to the quaternary ammonium group as the anionic binding site of the receptor in the case of drug-receptor interaction. The anions in the crystal structures can therefore be considered as a model for the receptor binding site. This concept can also be extended to cholinergic agonists which contain no trimethyl-ammonium group. Recently these results have been confirmed in principle. With a knowledge of our results and using the Cambridge Crystallographic Data File, Rosenfield and Murray-Rust have recently undertaken a systematic search for the cation-anion relationships in crystal structures containing a quaternary trimethylammonio methyl group [5, 6].

A differentiation of cholinergic activity into nicotinic and muscarinic mode can be achieved when one takes also into account the usually existing second binding site of the cholinergic cations, which is normally a partially negatively charged group. The triangles defined by the ammonium nitrogen, an anion occupying a face of type B, and the second partially charged group of the cation exhibit characteristic geometries for each activity mode.

Fig. 2 shows a typical geometry of a muscarinic "activity triangle" and Fig. 3 two mirror-equivalent ones for nicotinic activity. Of importance for the differentiation of activity is the angle between the N^+- anion vector and the vector connecting the charged groups of the cation. It should be mentioned that for muscarinic compounds there exists a second type of "activity triangle" formed with an ether or ester oxygen at the same atomic position as the ester oxygen in acetylcholine. The existence of two types of muscarinic "ac-

