Presence of Diosgenin in Tissue Cultures of *Dioscorea composita* Hemsl. and Related Species

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Abstract □ Four species of *Dioscorea* were grown as callus and suspension tissue cultures on revised Murashige and Skoog's tobacco medium and contained diosgenin.

Keyphrases □ Diosgenin, presence—tissue cultures, determination □ *Dioscorea* tissue cultures—diosgenin determination □ TLC—separation □ GLC—determination

A number of *Dioscorea* species (family *Dioscoreaceae*) are cultivated commercially for their tubers which yield diosgenin, an important starting compound for the manufacture of corticosteroidal drugs. Of these, only *D. composita* and *D. deltoidea* have previously been established as tissue cultures. Diosgenin, however, was not detected in the tissue cultures of *D. composita* (1–4) while the presence of diosgenin in the callus and suspension cultures of *D. deltoidea* is reported. In this communication, the authors report the establishment of four species of *Dioscorea* as tissue cultures and their diosgenin content.

EXPERIMENTAL

Tissue Culture—Callus cultures of *D. composita*, *D. deltoidea*, *D. floribunda*, and *D. spiculiflora* were initiated from aseptically germinated seedlings placed in glass vials containing revised Murashige and Skoog's tobacco medium supplemented with 1–3 p.p.m. 2,4-dichlorophenoxyacetic acid (2,4-D). Details of the cultural procedures followed were described previously (5). Yellowish-white to pale-brown callus developed from the seedling within 3–4 weeks, and these were subcultured to fresh agar media every 4 weeks. On low 2,4-D medium (1 p.p.m.), root primordia differentiated from *D. floribunda* callus tissues; therefore, all callus cultures (except *D. deltoidea*) were maintained on a medium containing 3 p.p.m. 2,4-D.

In the present investigation: (*a*) callus tissues which had undergone four subcultures and (*b*) later grown as suspension culture on a reciprocal shaker for 3 weeks were assayed for the presence of diosgenin. Differentiated and undifferentiated tissue cultures of *D. floribunda* were assayed separately for diosgenin.

Analysis—The dried and acid-hydrolyzed tissues were extracted with benzene for TLC (qualitative) (6) or with chloroform for GLC (quantitative). Dried chloroform extracts and standards (diosgenin and cholesterol) were silylated with a Tri-Sil–pyridine mixture (Pierce Chemical Co., Rockford, Ill.) and assayed (Varian Aerograph model 1740 with flame-ionization detector). A 1.52-m. × 0.63-cm. (5-ft. × 0.25-in.) glass column containing 80–100-mesh Varaport 30 coated with 3% OV-17 was used at 275°.

Table I—*D. deltoidea*—Strain I: Diosgenin Content of Suspension Cultures

Age, weeks	G.I.[a]	Medium, pH	Diosgenin, mg.% Dry Weight[b]
2	4.2	6.5	454.3
4	3.7	5.2	225.8
6	3.0	3.2	182.4
8	2.0	6.5	432.8
10	1.8	6.9	302.5

[a] Growth index (G.I.) = final dry weight/initial dry weight. [b] Average for cells obtained from three to five 500-ml. conical flasks containing 100 ml. RT.1 medium.

Table II—*Dioscorea* Tissue Cultures: Diosgenin Content and Growth Index

Species	Callus/ Diosgenin Content[a]	G.I.[b]	Suspension/ Diosgenin Contents[a]	G.I.[b]
D. deltoidea, Strain II	348.5	3.1	223.7	1.9
D. floribunda, undiff.	145.0	3.6	63.5	2.8
D. floribunda, diff.	94.5	3.0	65.0	2.2
D. spiculiflora	90.4	2.8	35.3	1.8
D. composita, Strain I	60.2	3.2	38.1	2.5
D. composita, Strain II	24.5	2.4	50.9	1.7

[a] Diosgenin content expressed as mg.% dry weight of 4-week-old callus tissue or 3-week-old first generation suspension tissue. [b] Growth index (G.I.) = final dry weight/initial dry weight.

RESULTS AND DISCUSSION

The quantitative assays for diosgenin from tissues and suspension cultures of different *Dioscorea* species are summarized in Tables I and II. Of the species examined, maximum diosgenin content was present in tissue cultures of *D. deltoida* and then in decreasing order of diosgenin production by callus tissue cultures in *D. floribunda* (undifferentiated), *D. spiculiflora*, and *D. composita*. Progressive changes in diosgenin content in *D. deltoidea* cell suspensions during the course of culture for 10 weeks were studied (Table I). The diosgenin content is high initially, declines, and again rises to a high level at 8 weeks. Of all the cultural parameters examined, the pH of the medium follows a strikingly similar pattern. However, further experimentation is needed to confirm and explain this observation and why *D. composita* was not reported earlier to contain diosgenin.

It has been suggested (7, 8) that diosgenin is biosynthesized principally in the aerial parts of *Dioscorea* species and is then translocated to the tubers where it is stored. Earlier studies (5, 6) clearly indicated that undifferentiated *D. deltoidea* tissue cultures produced diosgenin, whereas root-differentiated tissue cultures produced very small amounts. In this study, root-differentiated cultures of *D. floribunda* produced significant amounts of diosgenin.

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Role of Hydrophobic Interactions in Enzyme Inhibition by Drugs

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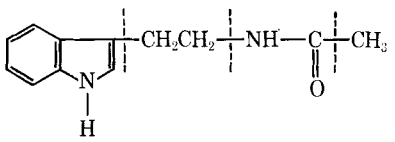
Abstract □ The role of hydrophobic interactions in inhibiting the relatively specific enzymatic reactions of five enzyme systems by series of congeneric drugs has been illustrated by the use of substituent constants and regression analysis. The inhibition of lipoxygenase by alcohols, the inhibition of D-amino acid oxidase by maleimides, and the inhibition of hydroxyindole-*o*-methyltransferase by *N*-acyltryptamines are found to be linearly dependent on the lipohydrophilic character of the inhibitors (log *P* or π). The inhibition of carbonic anhydrase by sulfonamides is found to be linearly dependent on the log *P* and Hammett's σ constant. For monoamine oxidase inhibition by substituted β-carbolines, a parabolic equation of log *P* gives the most significant correlation. The ideal lipohydrophilic character (log P_0) for maximum inhibition is found to be 2.74.

Keyphrases □ Enzyme inhibitory activity—hydrophobic interactions, drugs □ Hydrophobic interactions, drugs—enzyme inhibitory activity □ Physicochemical constants, enzyme inhibition—correlation

In recent years much effort has been focused on elucidation of weak intermolecular forces in biological systems (1), especially on the importance of hydrophobic interactions (2–6). Various experimental methods have been used to estimate the hydrophobic bonding tendency of drug molecules, such as partitioning and chromatographic methods (3, 7). Organic solvents capable of forming hydrogen bonds (*e.g.*, alcohols and esters) appear to give better correlations than hydrocarbons (8). The purpose of this paper is to correlate quantitatively enzyme inhibitory activity with the tendency of hydrophobic interactions of series of drugs, as measured by the partition coefficient of 1-octanol–water. It is hoped that this work may shed some light on the intermolecular forces involved in enzyme inhibition and provide some clues in designing new enzyme inhibitors.

METHOD

The biological data given in Table I are taken from the literature (4, 9–12). The Hammett's sigma constants (σ) are from the compilation of Jaffé (13) unless otherwise stated. The log *P* values are either experimentally determined or calculated from the π constants (14, 15) of Hansch. For example, the log *P* of *N*-acetyltryptamine is calculated as follows:



$$\Sigma\pi = 2.14 + 1.00 - 1.71 + 0.50 = 1.93 = \log P$$

The steric constants, E_s, are taken from Leffler and Grunwald (16). The equations listed in Table II are derived *via* the method of least squares using an IBM 360/65 computer. The inhibition constant K_I, $k' = (k_1/K_I)$, or the concentration of an inhibitor giving 50% inhibition of the enzyme (I_{50}) is converted to the molar basis, and pK_I, log k', or log $1/I_{50}$ is used as a measure of the inhibitory activity.

RESULTS

The equations correlating enzyme inhibition with the physicochemical constants are summarized in Table II, where *n* is the number of data points used in the analysis, *r* is the correlation coefficient, and *s* is the standard deviation.

In the inhibition of lipoxygenase by monohydric alcohols, the relative inhibitory activity is mainly determined by the lipohydrophilic character (log *P*). More than 98% ($r^2 = 0.983$) of the variance in the data can be accounted for by the simple linear equation (Eq. 1*a*). Equation 1*b*, derived by Mitsuda *et al.* (4), gives a slightly lower correlation coefficient, presumably due to the slightly different log *P* values used.

For the carbonic anhydrase inhibition by sulfonamides, by comparing Eq. 2*a* with Eq. 2*b* one can see that the electronic term σ is slightly more important than the log *P* term. The positive coefficient associated with σ indicates that electron-withdrawing groups will increase the inhibitory activity. By using both terms simultaneously, a much better correlation is obtained (Eq. 2*c*). The log *P* term in Eq. 2*c* is significant at 97.5-percentile level, as indicated by an *F*-test ($F_{1,16} = 7.2$; $F_{1,15\ 0.975} = 6.2$).

The π-constant alone gives almost perfect correlation for inhibition of D-amino acid oxidase by *N*-alkylmaleimides (Eqs. 3*b* and 3*c*). By using π and σ terms together, high correlation is obtained for the *N*-aryl as well as *N*-alkyl derivatives. The σ term in Eq. 3*a* is highly significant ($F_{1,5} = 111$; $F_{1,5\ 0.995} = 63.6$).

For the inhibition of hydroxyindole-*o*-methyltransferase by *N*-acyltryptamines, log *P* alone gives fairly good correlation (Eq. 4*a*). By deleting three molecules with deviation greater than 2*s*, a better