Isoflavonoid Phytoalexins of Yam Bean (*Pachyrrhizus erosus*)

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The furanopterocarpan, neodunol, has been isolated as a major phytoalexin from the fungus-inoculated stems of *Pachyrrhizus erosus*. Neodunol is accompanied by small quantities of demethylmedicarpin and a third compound provisionally identified as the prenylated pterocarpan, homoedudiol; traces of a neodunol isomer were also isolated from *P. erosus* although its precise structure could not be determined. The apparently close chemical relationship between *Pachyrrhizus* and *Neorautanenia* is discussed in the light of a recent study which allocates these genera to different sections of the legume tribe Phaseoleae.

Introduction

Pachyrrhizus erosus (L.) Urban (yam bean) is a climbing, papilionate legume indigenous to Mexico and northern Central America; the species has edible tubers and pods and is now widely cultivated in south-eastern Asia, India, Hawaii and parts of southern China. In early study, Norton and Hansberry [1] isolated the isoflavonoid fish poison rotenone from seeds of *P. erosus*. Subsequent examination led to the identification of a novel 3-phenylcoumarin (pachyrrhizin, **1**) [2] as well as numerous other isoflavonoids including isoflavanones (erosenone and neotenone) [3, 4], an isoflavone and a coumestan (dehydroneotenone and erosin respectively) [4, 5], and several rotenone analogues such as dolineone, erosone, pachyrrhizone and 12 a-hydroxyrotenone [3, 4, 6, 7]; surprisingly, in view of its remarkable chemical diversity, there is currently no evidence to suggest that seeds of *P. erosus* contain pterocarpan derivatives. It is noteworthy, however, that compounds belonging to this important isoflavonoid group are frequently found as phytoalexins [8] in the leaves, stems and hypocotyls of papilionaceous legumes [8, 9]. With the exception of rotenone and its 12 a-hydroxy analogue, all the abovementioned compounds possess a furano substituent at the same position as pachyrrhizin (**1**). In this respect, *P. erosus* resembles species belonging to the South African genus, *Neorautanenia* (*N. amboensis*, *N. edulis*, *N. ficifolia* and *N. pseudopachyrrhiza*) where furanoisoflavonoids − and particularly furanopterocarpans − are abundant [4, 10 − 16]. A number of recent studies have demonstrated that complex isoflavonoid phytoalexins are characteristically produced by several species (*e. g. Glycine max, Lablab niger, Phaseolus vulgaris, Psophocarpus tetragonolobus* and *Vigna unguiculata*) closely allied to *P. erosus* [8, 9]. An examination of the latter plant has now been undertaken and has revealed the presence of four pterocarpan derivatives none of which have previously been reported as legume phytoalexins. The isolation, identification and fungitoxic activity of these compounds is reported in this communication.

Results and Discussion

Etiolated stems of *P. erosus* were inoculated with spores of *Helminthosporium carbonum* [17] and subsequently extracted as described elsewhere [17, 18]. Si gel TLC ($CHCl_3$:MeOH, 50 : 1) [18] of these extracts gave four phenolic fractions (PE-1, R_F 0.61; PE-2, R_F 0.43; PE-3, R_F 0.19; and PE-4, R_F 0.05) which were eluted (EtOH) and re-chromatographed in *n*-pentane : Et_2O : HOAc, 75 : 25 : 1 (PE-1, R_F 0.52; PE-2, R_F 0.36) or 75 : 25 : 6, × 3 (PE-3/PE-4) prior to UV and MS analysis.

The major phytoalexin (PE-1; M^+ 280) had a UV (EtOH) spectrum which − apart from a peak at 294 nm − exhibited intense maxima at 248 and 255 nm reminiscent of those attributed to the furano substituent of neodulin (2,3-furano-8,9-methylenedioxypterocarpan, **2**), an extractive from the tubers of *Neorautanenia edulis* [10]. PE-1 formed both a monoacetate (M^+ 322) and a monomethyl ether (M^+ 294), the latter being indistinguishable (UV, MS, TLC) from authentic 9-*O*-methylneodunol (**3**). Hydrogenation yielded a phenolic tetrahydro derivative (**5**) with M^+ 284 and significant fragments at

1: R = H

16: R = OMe

2: R¹ = R² = O—CH₂—O

$2: R^1 = R^2 = O-CH_2-O$

$3: R^1 = H \; ; \; R^2 = OMe$

$4: R^1 = H \; ; \; R^2 = OH$
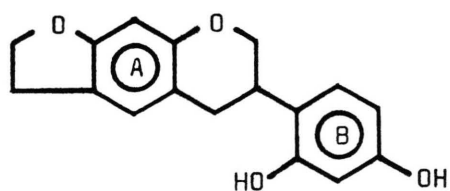
5

$6: R^1 = R^2 = H$

$7: R^1 = R^2 = Me$

$8: R^1 = H \; ; \; R^2 = Me$
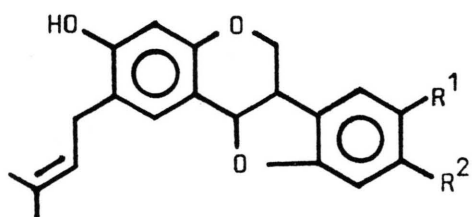
$9: R^1 = H \; ; \; R^2 = OH$
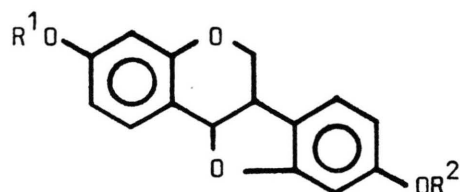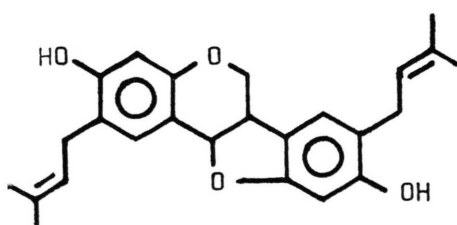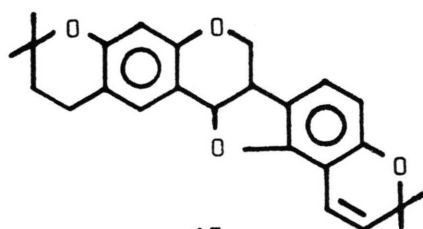
$10: R^1 = R^2 = O-CH_2-O$
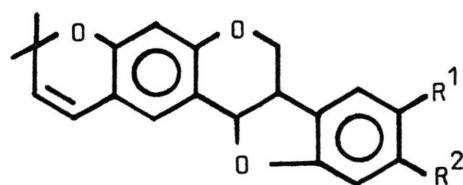
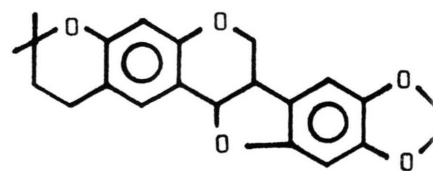$11: R^1 = H \; ; \; R^2 = OH$

$15: R^1 = R^2 = O-CH_2-O$

12

13

14

m/e 161 (dihydrofuran substituted A-ring) and 136 (dihydroxylated B-ring); as with neodulin (2), reduction of the furan ring eliminated the two low wavelength maxima (approx. 250 nm) characteristic of PE-1 [10]. All the above data suggested that PE-1 was identical with neodunol (2,3-furano-9-hydroxypterocarpan, 4) and this was confirmed by UV, MS and TLC comparison with a sample previously obtained from the root bark of *N. edulis* [11]. *Pachyrrhizus erosus* is only the second known source of this furanopterocarpan.

Compound PE-3 was readily characterised as 3,9-dihydroxypterocarpan (demethylmedicarpin, 6) by comparison (UV, MS, TLC) with authentic material [19]; methylation gave 3,9-dimethoxypterocarpan (homopterocarpin, 7). Demethylmedicarpin was initially obtained as a product resulting from metabolism of 3-hydroxy-9-methoxypterocarpan (medicarpin, 8) by the grey mould fungus, *Botrytis cinerea* [19]. Recently, however, small quantities of 6 have been isolated from leaf diffusates [19] of *Erythrina crista-galli* (tribe Phaseoleae; subtribe Erythrininae) and *E. insignis* following inoculation with *H. carbonum*, an organism which, in contrast to *B. cinerea*, apparently cannot demethylate medicarpin [19, 20]. These legumes also produce substantial amounts of 3,9-dihydroxy-10-isopentenylpterocarpan (phaseollidin) and it is conceivable, therefore, that demethylmedicarpin may be a biosynthetic precursor of this rare phytoalexin. In *P. erosus*, neodunol – and perhaps compounds PE-2/PE-3 (see below) – might similarly arise from 6.

As mentioned earlier, two other phytoalexins (PE-2 and PE-4) were also isolated from stems of *P. erosus*. Neither compound has been fully characterised although available spectroscopic data are given in the Experimental section. The MS of PE-2 had M^+ 324 (*cf.* phaseollidin [21]) and exhibited a major fragment ($M^+ - 55$; m/e 269) consistent with loss of isobutene from an isopentenyl substituent. Methylation afforded a dimethyl ether (M^+ 352). These data suggest that PE-2 (which is spectroscopically (UV) distinct from phaseollidin [20, 21]) may be identical with 2-isopentenyl-3,9-dihydroxypterocarpan (homoedudiol, 9), an isoflavonoid which co-occurs with neodunol in the root bark of *N. edulis* [11]. Unfortunately, it has not been possible to obtain an authentic sample of 9 for comparison with the *Pachyrrhizus* phytoalexin. Finally, the fourth phytoalexin (PE-4; M^+ 280) appears to be an isomer of neodunol. Both

compounds have virtually identical UV spectra (see Experimental) whilst methylation of PE-4 affords a product indistinguishable (UV, MS, TLC) from 9-*O*-methylneodunol (3). However, neither PE-2 nor PE-4 could be extracted in quantities sufficient to permit more detailed structural analyses; full characterisation of these compounds is not envisaged at the present time.

When incorporated into agar and tested against the mycelial growth of *H. carbonum*, neodunol (log $\epsilon = 4.34$ at 246 nm [11]) had an ED_{50} value of 27 µg/ml. Corresponding data for five other fungi were as follows: *Alternaria brassicicola* (ED_{50} 44 µg/ml), *Phoma betae* (ED_{50} 36 µg/ml), *P. lingam* (ED_{50} 51 µg/ml), *Monilinia fructicola* (ED_{50} 21 µg/ml) and *Botrytis cinerea* (ED_{50} approx. 100 µg/ml). The low ED_{50} value recorded for *B. cinerea* may reflect the fact that this fungus, which is pathogenic on numerous legumes, has the known capacity to detoxify pterocarpan phytoalexins [19, 22]. In a TLC bioassay using *Cladosporium herbarum* [17], neodunol (10 and 20 µg) gave inhibition zones of 94 and 226 mm² respectively. Of the other *Pachyrrhizus* phytoalexins, demethylmedicarpin has previously been found to inhibit the mycelial growth of *H. carbonum* [19]; similarly, although the fungitoxicity of PE-2 and PE-4 was not precisely determined, regions of slight antifungal activity corresponding to the location of these compounds were obtained when stem tissue extracts were subjected to TLC bioassay [17].

As shown in Table I, the *H. carbonum*-inoculated stems of *P. erosus* contained substantial quantities of

Table I. Concentration (µg/g fr. wt.) of pterocarpan phytoalexins in stems of *P. erosus* following treatment with *H. carbonum* or de-ionised H_2O.

Pterocarpan	Concentration [a, b, c]
Neodunol (PE-1)	374 – 487 (TR – 11)
Compound PE-2	3 – 7 (–)
Demethylmedicarpin (PE-3)	5 – 11 (–)
Compound PE-4	8 – 14 (–)

TR, trace; –, not detectable.

[a] Values indicate upper and lower concentration limits of five determinations involving tissue samples of between 1.5 and 2.5 g fr. wt.

[b] Concentrations (after 48 h incubation) of neodunol and PE-4 are calculated using the extinction coefficient for 4 (log = 4.34 at 246 nm [11]). Values for PE-2 and demethylmedicarpin are based respectively on log ϵ for phaseollidin (3.78 at 286.5 nm [21]) and medicarpin (3.89 at 287 nm [19]).

[c] Figures in parentheses refer to phytoalexin levels in stems treated with deionised H_2O.

neodunol but only trace amounts of the other three pterocarpan phytoalexins. All four compounds were absent from, or present at very low levels in, stems treated with de-ionised H_2O. There was no evidence to indicate that stem extracts contained rotenone (which was available as a chromatographic marker throughout this study) or any of the other isoflavonoids reported to occur in seeds of *P. erosus* [1 − 7].

Various pterocarpans including neodunol apparently occur constitutively in the tubers and root bark of *Neorautanenia edulis* [10, 11, 23 − 25]; several more have been obtained from the underground parts of other *Neorautanenia* species [14, 25, 26]. Whilst the exact function of these compounds remains obscure, it is possible that some may act by protecting the roots from invasion by soil-borne micro-organisms. Indeed, it is worth speculating if biosynthesis of certain minor *Neorautanenia* isoflavonoids (*e. g.* 4 and 9) [11, 27] is stimulated by the activities of root-inhabiting fungi, in which case these compounds could conceivably be regarded as phytoalexins. Interestingly, therefore, three additional phenolic *Neorautanenia* pterocarpans namely, edunol (10), neorautenol (11) and ficifolinol (12) [11, 23, 26], were found to be highly fungitoxic when 20 µg of each was tested (TLC bioassay) against *C. herbarum* [20]. In contrast, at a similar level, four non-phenolic pterocarpans (folinin (13), neodulin (2), neorautane (14) and neorautenane (15)) [10, 24, 26] from the same genus were inactive [20] suggesting that some degree of molecular hydroxylation may be an important prerequisite for fungitoxicity. Perrin and Cruickshank [28] also noted that neodulin lacked antifungal activity.

Although *P. erosus* is the only legume currently known to produce furanopterocarpan phytoalexins, a dihydrofuranoid analogue (glyceollin III) has recently been isolated from soybean (*Glycine max*) cotyledons treated with aqueous $CuCl_2$ [29]. Indeed, apart from the New World genus *Pachyrrhizus*, furanoisoflavonoids such as 4 have only been associated with species belonging to the Old World group, *Neorautanenia*, a fact which, despite their disjunct distribution, suggests that these two genera may be very closely related. In a recent study, *Pachyrrhizus* and *Neorautanenia* were allocated to different subtribes (Diocleinae and Glycininae respectively) of the tribe Phaseoleae *sensu lato* [30]. However, despite occurrence of the non-protein amino acid canavanine in seeds of *P. erosus* and most other Diocleinae and its

corresponding absence from the majority of Glycininae genera including *Neorautanenia* [30, 31], the abovementioned separation appears inconsistent when considered in terms of isoflavonoid chemistry. Thus, as stated earlier, furanoisoflavonoids in general and 3-phenylcoumarin derivatives in particular (pachyrrhizin (1) and neofolin (16) are the only reported examples of this latter group [2, 4, 16]) are quite exceptionally rare and to date have been recorded only in *Pachyrrhizus* and *Neorautanenia*. As compounds of this type are unlikely to have arisen spontaneously in these genera, it is logical to infer that *Pachyrrhizus* and *Neorautanenia* are more closely related than Lackey [30] and Bell *et al.* [31] have proposed.

Furthermore, it has been found that *P. erosus* produces a complex prenylated phytoalexin (PE-2) provisionally identified as homoedudiol (9). In contrast, a detailed examination of species belonging to several other genera (*Dioclea, Canavalia, Camptosema* and *Galactia*) assigned to the subtribe Diocleinae [30, 31] has revealed that all characteristically accumulate pterocarpans (*e. g.* 8) lacking the various complex substituents which frequently are associated with the subtribes Glycininae (*e. g.* glyceollins I − IV of *Glycine*) and Erythrininae/Phaseolinae (*e. g.* phaseollidin of *Erythrina, Phaseolus* and *Lablab* amongst others) [8, 9, 20, 29, 32]. Again, this evidence tends to support the view that *Pachyrrhizus* should be removed from the Diocleinae and placed within the Glycininae where its apparent relationship to *Glycine* and particularly *Neorautanenia* − an extremely rich source of prenylated pterocarpans [11, 23, 25, 26] − can be more fully appreciated.

Experimental

MS/UV analyses and all chromatographic separations were undertaken as previously described [18, 19]. Seeds of *P. erosus* were supplied by the International Institute of Tropical Agriculture, Ibadan, Nigeria.

Compound PE-1 (neodunol, 4). Diazotised *p*-nitroaniline, orange; Gibbs reagent, no reaction. λ max (nm) EtOH 213 (100%), 224 sh (77%), 241 (36%), 248 (42%), 255 (44%), 288 sh (39%), 294 (43%), 303 sh (28%); EtOH + NaOH 216 (100%), 240 sh (27%), 248 (32%), 255 (29%), 301 (21%). MS (rel. int.) 281 (15), 280 (M^+; 100), 279 (20), 263 (5),

171 (11), 158 (12), 149 (7), 147 (28), 140 (7), 134 (15), 123 (6), 111 (9), 109 (8), 107 (6), 105 (6). *Monomethyl ether* (**3**) (CH_2N_2; R_F 0.86, $CHCl_3 : CCl_4$, 3 : 1) λ max (nm) EtOH 212 (100%), 223 sh (86%), 241 (39%), 248 (47%), 255 (49%), 287 sh (42%), 293 (47%), 303 sh (27%). MS (rel. int.) 295 (53), 294 (M$^+$; 100), 293 (50), 279 (39), 277 (6), 265 (10), 171 (16), 165 (6), 161 (7), 158 (17), 148 (56), 147 (18), 133 (10), 115 (6). *Monoacetate* (Py − Ac$_2$O; R_F 0.74, $CHCl_3$) λ max (nm) EtOH 212 (100%), 224 sh (84%), 241 (38%), 248 (47%), 255 (50%), 284 sh (33%), 290 (36%), 303 sh (24%). MS (rel. int.) 323 (4), 322 (M$^+$; 23), 280 (100), 279 (26), 263 (6), 171 (16), 165 (14), 158 (18), 152 (11), 147 (33), 134 (21).

Tetrahydroneodunol (**5**). Neodunol (**4**) from *P. erosus* (approx. 1 mg) was hydrogenated as described elsewhere [33]. Work up and Si gel TLC ($CHCl_3 : MeOH$, 50 : 1) gave a small quantity (approx. 150 µg) of **5** (R_F 0.21; *cf.* **4**, R_F 0.61). Diazotised *p*-nitroaniline, yellow; Gibbs reagent, purple/blue. λ max (nm) EtOH 214 (100%), 258 sh (10%), 283 sh (30%), 289 (35%), 293 sh (34%), 299 sh (31%), 304 sh (25%); EtOH + NaOH 210 (100%), 244 sh (7%), 295 sh (8%), 299 (9%), 302 sh (6%). MS (rel. int.) 284 (M$^+$; 11), 161 (8), 150 (10), 149 (52), 136 (18), 135 (17), 133 (5), 121 (4), 107 (24), base peak at *m/e* 43.

Compound PE-2 (*homoedudiol* (*?*), **9**). Diazotised *p*-nitroaniline, orange; Gibbs reagent, no reaction. λ max (nm) EtOH 213 (100%), 230 sh (50%), 284 sh (31%), 288 (34%), 293 sh (32%); EtOH + NaOH 216 (100%), 249 (30%), 301 (23%). MS (rel. int.)

325 (7), 324 (M$^+$; 38), 323 (6), 269 (32), 268 (8), 149 (11), 147 (11), 123 (8), 111 (10), 109 (8), base peak at *m/e* 43. *Dimethyl ether* (R_F 0.83, $CHCl_3 : CCl_4$, 3 : 1) λ max (nm) EtOH 213 (100%), 230 sh (49%), 282 sh (26%), 287 (29%), 291 sh (28%). MS (rel. int.) 352 (M$^+$; 100), 337 (5), 297 (8), 162 (6), 161 (19), 149 (15), 148 (14), 137 (9), 133 (5).

Compound PE-3 (*demethylmedicarpin*, **6**). Diazotised *p*-nitroaniline, orange; Gibbs reagent, no reaction. UV (EtOH; EtOH + NaOH) and MS as lit. [19]. *Dimethyl ether* (**7**) (R_F 0.84, $CHCl_3 : CCl_4$, 3 : 1) UV and MS as lit. [19].

Compound PE-4 (*'isoneodunol'*). Diazotised *p*-nitroaniline, orange; Gibbs reagent, no reaction. λ max (nm) EtOH 211 (100%), 224 sh (65%), 240 sh (28%), 248 (30%), 255 (31%), 288 sh (25%), 294 (28%), 304 sh (18%); EtOH + NaOH 220 (100%), 241 sh (24%), 248 (27%), 256 (24%), 302 (19%). MS (rel. int.) 281 (15), 280 (M$^+$; 100), 279 (36), 171 (13), 158 (16), 149 (43), 147 (33), 134 (19), 125 (8), 123 (10), 119 (7), 118 (7), 111 (18), 109 (15), 105 (7). *Monomethyl ether* TLC, UV and MS data as given for neodunol methyl ether (**3**).

[1] L. B. Norton and R. Hansberry, J. Amer. Chem. Soc. **67**, 1609 (1945).
[2] E. Simonitsch, H. Frei, and H. Schmid, Mh. Chem. **88**, 541 (1957).
[3] A. J. Kalra, M. Krishnamurti, and M. Nath, Indian J. Chem. **15 B**, 1084 (1977).
[4] L. Crombie and D. A. Whiting, J. Chem. Soc. **1963**, 1569.
[5] J. Eisenbeiss and H. Schmid, Helv. Chim. Acta **42**, 61 (1959).
[6] M. Krishnamurti, Y. Sambhy, and T. R. Seshadri, Tetrahedron **26**, 3023 (1970).
[7] H. Bickel and H. Schmid, Helv. Chim. Acta **36**, 664 (1953).
[8] J. B. Harborne and J. L. Ingham, Biochemical Aspects of Plant and Animal Coevolution (J. B. Harborne ed.), p. 343, Academic Press, London and New York 1978.
[9] J. L. Ingham, Proc. Int. Legume Conf. Kew 1978, in press.
[10] B. L. Van Duuren, J. Org. Chem. **26**, 5013 (1961).

[11] A. J. Brink, G. J. H. Rall, and J. P. Engelbrecht, Phytochemistry **13**, 1581 (1974).
[12] M. E. Oberholzer, G. J. H. Rall, and D. G. Roux, Tetrahedron Letters **1974**, 2211.
[13] M. E. Oberholzer, G. J. H. Rall, and D. G. Roux, Phytochemistry **15**, 1283 (1976).
[14] M. E. Oberholzer, G. J. H. Rall, and D. G. Roux, Tetrahedron Letters **1977**, 1165.
[15] C. v. d. M. Brink, J. J. Dekker, E. C. Hanekom, D. H. Meiring, and G. J. H. Rall, J. S. African Chem. Inst. **18**, 21 (1965).
[16] C. v. d. M. Brink, W. Nel, G. J. H. Rall, J. C. Weitz, and K. G. R. Pachler, J. S. African Chem. Inst. **19**, 24 (1966).
[17] J. L. Ingham, Phytopathol. Z. **87**, 353 (1976).
[18] J. L. Ingham, Z. Naturforsch. **31 c**, 504 (1976).
[19] J. L. Ingham, Phytochemistry **15**, 1489 (1976).
[20] J. L. Ingham, unpublished data.
[21] D. R. Perrin, D. R. Biggs, and I. A. M. Cruickshank, Aust. J. Chem. **27**, 1607 (1974).

[22] J. v. d. Heuvel and J. A. Glazener, Neth. J. Pl. Path. **81,** 125 (1975).

[23] G. J. H. Rall, J. P. Engelbrecht, and A. J. Brink, J. S. African Chem. Inst. **24,** 56 (1971).

[24] G. J. H. Rall, A. J. Brink, and J. P. Engelbrecht, J. S. African Chem. Inst. **25,** 25 (1972).

[25] A. J. Brink, G. J. H. Rall, and J. C. Breytenbach, Phytochemistry **16,** 273 (1977).

[26] C. v. d. M. Brink, J. P. Engelbrecht, and D. E. Graham, J. S. African Chem. Inst. **23,** 24 (1970).

[27] A. J. Brink, G. J. H. Rall, and J. P. Engelbrecht, Tetrahedron **30,** 311 (1974).

[28] D. R. Perrin and I. A. M. Cruickshank, Phytochemistry **8,** 971 (1969).

[29] R. L. Lyne, L. J. Mulheirn, and D. P. Leworthy, J. Chem. Soc. Chem. Commun. **1976,** 497.

[30] J. A. Lackey, J. Linn. Soc. (Bot.) **74,** 163 (1977).

[31] E. A. Bell, J. A. Lackey, and R. M. Polhill, Biochem. Syst. Ecol. **6,** 201 (1978).

[32] R. L. Lyne and L. J. Mulheirn, Tetrahedron Letters **1978,** 3127.

[33] J. L. Ingham, Z. Naturforsch. in press.