A Chemical Investigation of *Pueraria mirifica* Roots

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Ten isoflavonoids including daidzein, daidzin (daidzein-7-O-glucoside), puerarin (daidzein-8-C-glucoside), genistein and coumestrol have been isolated from a methanolic extract of *Pueraria mirifica* roots. Apart from these known compounds and mirificin, a novel apioside derivative of puerarin, the roots have also been found to contain three minor coumestans and one 5-deoxyisoflavone for which only limited spectroscopic data are currently available.

Introduction

The flowers and/or below ground parts of three species (*Pueraria thunbergiana* = *P. lobata, P. montana* and *P. tuberosa*) belonging to the Asian legume genus *Pueraria* (Leguminosae-Papilionoideae; tribe Phaseoleae) have proved to be extremely rich in isoflavonoid compounds, and more than 25 structures have now been reported [1–5]. These compounds are predominantly isoflavones although increasingly other types of isoflavonoid (*e.g.* the coumestan derivatives coumestrol and tuberostan from *P. thunbergiana* stem callus [2] and *P. tuberosa* roots [4] respectively) are being discovered in *Pueraria* tissues. In addition to tuberostan, for example, the tubers of *P. tuberosa* contain the fungitoxic and anti-tubercular pterocarpan tuberosin [6] as well as a related pterocarpanone (hydroxytuberosone) and two pterocarpenes (anhydrotuberosin and 3-O-methylanhydrotuberosin) [3, 4]. Tuberosin and a second pterocarpan, glycinol, also accumulate as phytoalexins [7] in the fungus-inoculated leaflets of *P. thunbergiana* [1]. Both 5-oxy (*e.g.* genistein and biochanin A) and 5-deoxy (*e.g.* daidzein and formononetin) isoflavones occur in *Pueraria* species [1], and these may be further substituted with either O- or C-linked sugars. The isoflavone C-glucoside puerarin (daidzein-8-C-glucoside, **4**) [8] and its various derivatives (4′,6″-di-O-acetylpuerarin, 3′-hydroxypuerarin, 3′-methoxypuerarin and "puerarin xyloside") [8–10], and the O-glycoside kakkalide [11] are apparently unique to *Pueraria,* as also is the isoflavone aglycone kakkatin [12].

As yet, there are no reports of isoflavonoids from *P. mirifica* Airy Shaw & Suvatabandhu [13], an exceptionally rare woody climber with globular or tapering tuberous roots native to the forests of northern Thailand where it is known locally as kwao khua (kwao keur in ref. [13]). However, *P. mirifica* roots are known to contain a potent oestrogenic compound (miroestrol, **1**) [14–17] somewhat similar in structure to the isoflavone daidzein (7,4′-dihydroxyisoflavone, **2**), a weak oestrogen [18] already found in other *Pueraria* species [1, 2]. The occurrence of **1** in *P. mirifica* suggested that this plant might also be a source of known or hitherto unrecognized isoflavonoid structures with useful physiological properties. In this paper, we report the results of a recent re-examination of the dried *P. mirifica* root material remaining from the original miroestrol study carried out between 1952 and 1960 [14, 19]. Apart from daidzein and its 7-O-glucoside (daidzin), the roots have yielded puerarin, genistein and coumestrol in addition to the unique puerarin derivative, mirificin (puerarin-6″-O-β-apiofuranoside) [20]. Four other apparently new isoflavonoids, an isoflavone aglycone and three coumestans, were also isolated at the same time, but the small quantities available for chemical analysis have prevented their complete characterization.

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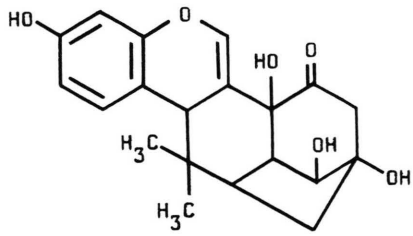

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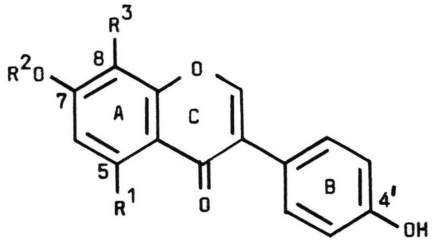

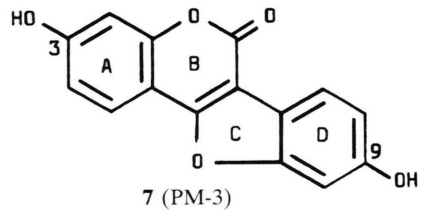

7 (PM-3)

2 (PM-5) : $R^1 = R^2 = R^3 = H$
3 (PM-8) : $R^1 = R^3 = H$; R^2 = Glucose
4 (PM-9) : $R^1 = R^2 = H$; R^3 = Glucose
5 (PM-10): $R^1 = R^2 = H$; R^3 = Glucose-Apiose
6 (PM-2) : R^1 = OH; $R^2 = R^3 = H$

Results and Discussion

A methanolic extract of dried *P. mirifica* root was chromatographed (Si gel preparative TLC, layer thickness 0.5 mm) in $CHCl_3$–MeOH (20:1) to afford six major bands (R_F 0.52, 0.33, 0.27, 0.22, 0.18 and 0.11) all of which appeared blue or pale-blue under long wavelength (365 nm) UV light. The origin region of the chromatogram (R_F 0.00–0.05) also exhibited a blue fluorescence and, like that of the bands at R_F 0.22 and 0.11, this colour greatly intensified upon fuming for approx. 20 sec with NH_3 vapour. After elution with MeOH, the components of each band were further purified by Si gel TLC (layer thickness 0.25 mm) as described in the Experimental section to give ten chromatographically homogeneous compounds which were designated PM-1 ~ PM-10.

Compounds PM-2, PM-3 and PM-5 were readily identified as genistein (5,7,4′-trihydroxyisoflavone, **6**), coumestrol (3,9-dihydroxycoumestan, **7**) and daidzein (7,4′-dihydroxyisoflavone, **2**) respectively by UV and Si gel TLC comparison (3 solvent systems) with authentic reference samples of either natural (**7**) or synthetic (**2** and **6**) origin. Upon hydrolysis with boiling 2 N HCl for 30 min [21] compound PM-8 afforded daidzein (**2**; identified by comparative UV spectroscopy and Si gel TLC) and a monosaccharide inseparable by paper chromatography in *n*-BuOH–Bz–Py–H_2O (5:1:3:3) from a glucose standard (R_F approx. 0.31). Although PM-8 gave an orange colour on thin-layer plates sprayed with diazotized *p*-nitroaniline reagent (indicative of an aromatic OH group), its methanolic UV spectrum was unaffected by NaOAc (C-7 OH derivatized [21]). Compound PM-8 was therefore provisionally formulated as daidzein-7-O-glucoside (daidzin, **3**), a view subsequently confirmed by comparison (UV, Si gel TLC) with a sample of daidzin previously obtained from the stem callus of *P. thunbergiana* [2].

On thin-layer plates developed in $CHCl_3$–MeOH (20:1), daidzin (R_F 0.00–0.05) co-chromatographed with two other pale blue fluorescent isoflavones (PM-9 and PM-10) but all three compounds were eventually resolved by TLC in $CHCl_3$–MeOH–H_2O

(20:10:1; see Experimental for R_F values). One of these isoflavones (PM-9) was readily identified as puerarin (daidzein-8-C-glucoside, **4**), a well known *Pueraria* root constituent [1], following comparison (UV, PC and Si gel TLC) with material originally derived from *P. thunbergiana* [2]. In contrast to PM-9, however, compound PM-10 proved to be a new isoflavone C-glycoside, affording puerarin and the rare furanoside apiose upon treatment with boiling 2 N HCl [21]. From a consideration of its ^{1}H and ^{13}C NMR spectra [20] this isoflavone, which we have decided to call mirificin, was identified as puerarin-6″-O-β-apiofuranoside (**5**). Mirificin is unique amongst the relatively few naturally occurring flavonoid and isoflavonoid apioglucosides in that the sugar residue is characterized by a 1 → 6, apiose to glucose, link [20]. In related apioglucosides such as the flavone apiin from *Petroselinum crispum* (Umbelliferae) [22] and the isoflavone lanceolarin from *Dalbergia lanceolaria* (Leguminosae) [23], the apiose and glucose residues have been found to be 1 → 2 linked.

In addition to the compounds already mentioned, *P. mirifica* roots were found to contain four other isoflavonoid derivatives (PM-1, PM-4, PM-6 and PM-7) although none of these was obtained in quantities sufficient for complete structure elucidation. However, from their UV (MeOH; MeOH + NaOH; MeOH + NaOAc) spectra, it was clear that, like coumestrol (**7** = PM-3), compounds PM-1, PM-4 and PM-6 were all coumestans possessing an A-ring hydroxyl group at C-3 (NaOAc-induced bathochromic shift of the 347 nm MeOH maximum [24]). Moreover, whilst attempts to obtain MS data for PM-4 and PM-6 were unsuccessful, PM-1 readily afforded fragments [M^+ 366, *m/z* 351 ($M^+ - 15$), 311 ($M^+ - 55$)] consistent with a molecule containing two OH groups, a single OCH_3 substituent and an isopentenyl (3,3-dimethylallyl) sidechain. Compound PM-1 at least would therefore appear to be a new natural product since the only comparably substituted coumestan (glycyrol from the root of a *Glycyrrhiza* species [1, 25]) carries a methoxyl group at C-3, and thus gives a UV (MeOH) spectrum that is unaffected by addition of NaOAc [25].

From its UV (MeOH) spectrum and characteristic fluorescence on thin-layer plates viewed under long wavelength UV light (pale blue, intensifying upon exposure to NH_3 vapour), the final *Pueraria* isoflavonoid (PM-7; $[M]^+$ 368) was identified as a 5-deoxyisoflavone. The presence of an A-ring substituted as in daidzein (**2**) was apparent from the UV shift induced by NaOAc (C-7 OH [21]), and from the prominent fragments observed at *m/z* 137 ($C_7H_5O_3$) and *m/z* 151 ($C_8H_7O_3$) respectively in the mass spectrum of the parent isoflavone and its non-phenolic trimethyl derivative ($[M]^+$ 410). Signals at δ 8.02 (d, 1H, J = 8.5 Hz), 6.98 (dd, 1H, J = 8.5 & 2.4 Hz) and 6.91 (d, 1H, J = 2.4 Hz) in the ^{1}H NMR spectrum (acetone-d_6; 500 MHz) of PM-7 were also consistent with monohydroxylation of ring A (*cf.* chemical shift values obtained in DMSO-d_6 for H-5, δ 7.94d, J = 9.0 Hz; H-6, δ 6.90dd, J = 9.0 & 2.0 Hz; and H-8, δ 6.83d, J = 2.0 Hz of daidzein from *P. thunbergiana* [2]). Apart from a ^{1}H NMR signal attributable to H-2 (δ 7.84s, 1H), the spectrum of PM-7 also revealed an aromatic proton singlet (δ 6.39), a methoxyl group (δ 3.71s, 3H), and a 3,3-dimethylallyl sidechain (δ 1.39s and 1.49s, both 3H, 2 × C<u>H</u>$_3$; 5.01br t, J = *ca* 6.7 Hz, C<u>H</u>; 3.09dd, J = 14.7 & 7.3 Hz and 3.31dd, J = 14.7 & 6.1 Hz, C<u>H</u>$_2$). By deduction, the alkenyl sidechain, the OCH_3 group and the lone proton were assigned to ring B which, as in all previously reported *Pueraria* isoflavones [1, 2, 5], can be assumed to have an oxygen function at C-4′. Unfortunately, however, a lack of PM-7 for chemical investigation has thusfar prevented the relative positions of the B-ring substituents from being precisely defined.

No evidence was obtained to indicate that *P. mirifica* roots contained the pterocarpan tuberosin [6] or many of the isoflavone aglycones (*e.g.* formononetin, biochanin A and irisolidone) and O-glycosides (*e.g.* ononin, sissotrin and tectoridin) previously found elsewhere in the genus *Pueraria* [1]. Although it is possible that tuberosin, a comparatively labile 6a-hydroxy pterocarpan, may have decomposed during drying and subsequent prolonged storage of the roots, the apparent absence of known isoflavones other than daidzein (**2**), daidzin (**3**), puerarin (**4**) and genistein (**6**) suggests that these normally stable compounds are either not produced by *P. mirifica,* or that in the root tissues they occur only as trace constituents.

Several of the isoflavonoids identified during the present study are known to be physiologically and pharmacologically active in man and other animals. Thus daidzein and genistein both exhibit weak oestrogenic properties when administered orally to mice whilst coumestrol is considerably more potent

[18]. Coumestrol and genistein are also oestrogenic in ruminants (ewes) [24], but additionally may strongly inhibit the action of potent oestrogens such as oestradiol-17β, oestrone and diethylstilboestrol in immature mice [26, 27]. The presence of the three isoflavone glycosides daidzin, puerarin and mirificin might in part explain the observation by Jones and Pope [19] that considerable oestrogenic activity was associated with aqueous solutions of *P. mirifica* root extracts from which all, or virtually all, the miroestrol (**1**) had been removed with diethyl ether. Other physiological attributes of *Pueraria* isoflavones include the relaxing (antispasmodic) effect of daidzein on contracting smooth muscle from mouse gut [28], and the demonstration that this compound and its 7-O- and 8-C-glucosides (daidzin and puerarin respectively) are effective in relieving headaches [29]. The importance of *Pueraria* species, including *P. mirifica,* in the traditional medicine of India [9] and other Asian countries (Thailand, Burma, China and Japan) [8, 13, 14] strongly suggests that this legume genus may be a source of novel isoflavonoids, or other natural products, possessing useful physiological properties.

Experimental

Plant material

Roots of *Pueraria mirifica* Airy Shaw & Suvatabandhu were collected in northern Thailand from forest sites near the town of Chiangmai. The roots were sliced and air-dried, the pieces then being shipped to Reading for storage in sealed glass containers at room temperature until needed for investigation.

Extraction and purification of Pueraria isoflavonoids

A 30 g sample of dried *P. mirifica* root was finely powdered and then stirred for about 12 h in warm (40 °C) 95% aqueous MeOH (100 ml). After vacuum filtration, the solvent was concentrated to approx. 10 ml *in vacuo* (35 °C) and filtered again. The pale yellow filtrate was applied as a streak to several preparative Si gel thin-layer plates (Merck, F-254, layer thickness 0.5 mm) and these were then developed in $CHCl_3$–MeOH (20:1). Bands of Si gel at R_F 0.52 (*B1*), 0.33 (*B2*), 0.27 (*B3*), 0.22 (*B4*), 0.18 (*B5*), 0.11 (*B6*) and 0.05 – origin (*B7*) corresponding to the location of material fluorescing blue or pale blue under long wavelength (approx. 365 nm) UV light were removed and the compound(s) responsible were eluted with MeOH (25 ml). The eluates were reduced to dryness (*in vacuo,* 35 °C) and the residues were taken up in MeOH (1–1.5 ml) prior to Si gel TLC (Merck, F-254, layer thickness 0.25 mm) as follows: a) *B1* in *n*-pentane–Et_2O–glacial HOAc–MeOH (PEAM, 75:25:6:3) to give coumestan PM-1 at R_F 0.55; b) *B2, B3* and *B5* in PEAM (75:25:6:3, ×3–×5) to give respectively genistein (**6** = PM-2, upper zone) + coumestrol (**7** = PM-3, lower zone), coumestan PM-4, and coumestan PM-6; c) *B4* in *n*-hexane–acetone–MeOH (30:15:1, ×3) to give daidzein (**2** = PM-5); d) *B6* in PEAM (75:25:6:10) to give isoflavone PM-7 at R_F 0.24, and e) *B7* in $CHCl_3$–MeOH–H_2O (20:10:1) to give daidzin (**3** = PM-8), puerarin (**4** = PM-9) and mirificin (**5** = PM-10) at R_F 0.63, 0.53 and 0.37 respectively. Minor residual contaminants of coumestans PM-1, PM-4 and PM-6, and isoflavone PM-7 were finally removed by Si gel TLC in Bz–MeOH (BM) 9:1 (PM-1, R_F 0.27), BM, 20:3 (PM-4, R_F 0.27) or BM, 5:1 (PM-6, R_F 0.33; PM-7, R_F 0.26).

Characterization of Pueraria isoflavonoids

The structure, or part structure, assigned to each *Pueraria* isoflavonoid was deduced mainly by spectroscopic (UV in MeOH, or MeOH plus an appropriate shift reagent; see text and section below) and chromatographic (TLC on Si gel or PC on Whatman No. 1 paper) comparison with reference compounds, and in the case of glycosides PM-8 (daidzin), PM-9 (puerarin) and PM-10 (mirificin) by hydrolysis or attempted hydrolysis with boiling 2 N HCl [21] to afford an identifiable isoflavone and sugar residue. The pale blue long wavelength UV fluorescence (intensifying after exposure to NH_3 vapour) exhibited on Si gel thin-layer plates by PM-5, PM-7, PM-9 and PM-10 also suggested that these compounds were 5-deoxyisoflavones [21]. Reference samples of daidzein and genistein were purchased from Apin Chemicals Ltd., Abingdon, England. Daidzin and puerarin were kindly supplied by Dr. H. Itokawa (Tokyo College of Pharmacy, Tokyo, Japan), and a specimen of authentic coumestrol was obtained from Dr. E. M. Bickoff (Western Regional Research Laboratory, U.S. Dept. of Agriculture, Albany, California, U.S.A.).

Spectroscopic properties of Pueraria isoflavonoids

a) PM-1 (partially characterized coumestan). UV: λ_{max}, nm: MeOH 208, 254, 294, 306, 347, 362 sh; + NaOH 208, 268, 322, 388; + NaOAc 255, 280 sh, 312, 366, 382 sh (addition of boric acid regenerated the MeOH spectrum). MS: $[M]^+$ 366 (base peak), *m/z* 351 ($M^+ - 15$), 335, 321, 311 ($M^+ - 55$), 310, 309, 297, 296. b) PM-2 (genistein, **6**). UV data as lit. [21, 30]. c) PM-3 (coumestrol, **7**). UV: λ_{max}, nm: MeOH 210, 244, 266 sh, 293, 305, 344, 360 sh; + NaOH 207, 253 sh, 277, 318, 383; + NaOAc as lit. [24]. d) PM-4 (partially characterized coumestan). UV: λ_{max}, nm: MeOH 212, 254, 293 sh, 305, 347, 362 sh; + NaOH 210, 270, 320, 390; + NaOAc 255, 282 sh, 313, 366, 383 sh (addition of boric acid regenerated the MeOH spectrum). e) PM-5 (daidzein, **2**). UV: λ_{max}, nm: MeOH 212, 240 sh, 250, 263 sh, 306; + NaOH 215, 260, 292 sh, 332; + NaOAc 256, 273 sh, 312 sh, 337 (addition of boric acid regenerated the MeOH spectrum). f) PM-6 (partially characterized coumestan). UV: λ_{max}, nm: MeOH 212, 254, 293 sh, 306, 347, 362 sh; + NaOH 208, 268, 323, 394; + NaOAc 256, 282 sh, 313, 368, 383 sh (addition of boric acid regenerated the MeOH spectrum). g) PM-7 (partially characterized 5-deoxyisoflavone). UV: λ_{max}, nm: MeOH 210, 242 sh, 250, 293, 308 sh; + NaOH 210, 256, 300, 332; + NaOAc 253, 288, 332 (addition of boric acid regenerated the MeOH spectrum). MS: $[M]^+$ 368, *m/z* 353 ($M^+ - 15$), 313 ($M^+ - 55$), 297, 258, 244, 243, 217, 201, 169, 137 (base peak). *Trimethyl ether* (CH_2N_2; R_F 0.17 in $CHCl_3$). UV: λ_{max}, nm: MeOH 210, 242 sh, 249, 291, 306 sh. The MeOH spectrum was unaffected by aqueous NaOH. MS: $[M]^+$ 410, *m/z* 395 ($M^+ - 15$), 379 ($M^+ - 31$), 341, 339, 260, 259, 245, 190, 152, 151 (base peak). h) PM-8 (daidzin, **3**). UV: λ_{max}, nm: MeOH 208, 230 sh, 248 sh, 258, 304 sh. The MeOH spectrum was unaffected by NaOAc. i) PM-9 (puerarin, **4**). UV: λ_{max}, nm: MeOH 206, 243 sh, 251, 263 sh, 309; + NaOH 213, 265, 292 sh, 336; + NaOAc 260, 342 (addition of boric acid regenerated the MeOH spectrum). j) PM-10 (mirificin, **5**). UV: λ_{max}, nm: MeOH 208, 242 sh, 252, 263 sh, 308; + NaOH 210, 263, 293 sh, 336; + NaOAc 259, 340 (addition of boric acid regenerated the MeOH spectrum).

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[1] J. L. Ingham, Fortschr. Chem. Org. NatStoffe **43,** 1 (1983).
[2] K. Takeya and H. Itokawa, Chem. Pharm. Bull. Tokyo **30,** 1496 (1982).
[3] A. V. K. Prasad, A. Singh, R. S. Kapil, and S. P. Popli, Indian J. Chem. **23B,** 1165 (1984).
[4] A. V. K. Prasad, R. S. Kapil, and S. P. Popli, Indian J. Chem. **24B,** 236 (1985).
[5] M. Kikuchi, Ann. Rep. Tohoku Coll. Pharm. **29,** 61 (1982).
[6] B. S. Joshi and V. N. Kamat, J. Chem. Soc. Perkin Trans. I **1973,** 907.
[7] J. L. Ingham, Phytoalexins (J. A. Bailey and J. W. Mansfield, eds.), p. 21, Blackie, Glasgow 1982.
[8] T. Murakami, Y. Nishikawa, and T. Ando, Chem. Pharm. Bull. Tokyo **8,** 688 (1960).
[9] S. P. Bhutani, S. S. Chibber, and T. R. Seshadri, Indian J. Chem. **7,** 210 (1969).
[10] S. Natori, N. Ikegawa, and M. Suzuki, Experimental Methods of Organic Natural Products, p. 470, Kodansha Publishers, Tokyo 1977.
[11] T. Kurihara and M. Kikuchi, J. Pharm. Soc. Japan **95,** 1283 (1975).
[12] M. Kubo, M. Sasaki, K. Namba, S. Naruto, and H. Nishimura, Chem. Pharm. Bull. Tokyo **23,** 2449 (1975).
[13] L. Kashemsanta, K. Suvatabandhu, and H. K. Airy Shaw, Kew Bull. **1952,** 549.
[14] J. C. Cain, Nature **188,** 774 (1960).
[15] N. E. Taylor, D. C. Hodgkin, and J. S. Rollett, J. Chem. Soc. **1960,** 3685.
[16] D. G. Bounds and G. S. Pope, J. Chem. Soc. **1960,** 3696.
[17] J. M. Ohrt and D. A. Norton, Nature **201,** 1210 (1964).
[18] E. M. Bickoff, A. L. Livingston, A. P. Hendrickson, and A. N. Booth, J. Agric. Fd. Chem. **10,** 410 (1962).
[19] H. E. H. Jones and G. S. Pope, J. Endocrinol. **22,** 303 (1961).
[20] J. L. Ingham, K. R. Markham, S. Z. Dziedzic, and G. S. Pope, Phytochemistry, in press.
[21] T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer, Berlin 1970.
[22] R. Hemming and W. D. Ollis, Chem. Ind. **1953,** 85.
[23] A. Malhotra, V. V. S. Murti, and T. R. Seshadri, Tetrahedron **23,** 405 (1967).
[24] E. M. Bickoff, R. R. Spencer, S. C. Witt, and B. E. Knuckles, Studies on the Chemical and Biological Properties of Coumestrol and Related Compounds, U.S. Dept. Agric., Technical Bull. No. 1408, U.S. Govnt. Printing Office, Washington D.C. 1969.

[25] T. Saitoh and S. Shibata, Chem. Pharm. Bull. Tokyo **17,** 729 (1969).
[26] Y. Folman and G. S. Pope, J. Endocrinol. **34,** 215 (1966).
[27] Y. Folman and G. S. Pope, J. Endocrinol. **44,** 213 (1969).
[28] S. Shibata, T. Murakami, Y. Nishikawa, and M. Harada, Chem. Pharm. Bull. Tokyo **7,** 134 (1959).
[29] C. C. Fang, M. Lin, C. M. Sun, H. M. Liu, and H. Y. Lang, Chung-Hua I Hsueh Tsa Chih **54,** 271 (1974); Chem. Abstr. **82,** 47672 (1975).
[30] J. L. Ingham, Z. Naturforsch. **31c,** 504 (1976).