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PHYTOCHEMICAL STUDIES OF THE CHINESE HERB TAI-ZI-SHEN, *PSEUDOSTELLARIA HETEROPHYLLA*[1]

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ABSTRACT.—The lipid fraction of *Pseudostellaria heterophylla* contains, in addition to palmitic and linoleic acid, the 1-monoglyceride of the latter and a new compound that was deduced from its spectral properties (ms, 1D and 2D ^{1}H- and ^{13}C-nmr spectra) and proved by synthesis to be 3-furfuryl pyrrole-2-carboxylate [**1**]. The complete nmr assignments of this compound were determined.

As part of our continuing interest in the chemistry of Chinese herbal medicines (1–3), we have undertaken a phytochemical examination of the roots of *Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm. (Caryophyllaceae), which is also known as t'ai-tzu-shen, tai-zi-shen, hai-erh-shen, taishisan, or lesser ginseng (4). This drug has a long history of use in Chinese herbal medicine as a pediatric and geriatric tonic in much the same way that ginseng (*Panax ginseng*) is used by adults (5). In contrast to the extensive investigations of ginseng, however, very little research has been carried out on this drug.

Japanese researchers (6) have examined the amino acid and sugar content of several *Pseudostellaria* species including *P. heterophylla*, while a Chinese group (7) has determined its trace element composition. Korean investigators have included a related plant, *Pseudostellaria pallibiniana*, in their phytochemical survey of the Caryophyllaceae (8) and have recently isolated therefrom isovitexin, a flavone-*C*-glycoside (9). The importance of *P. heterophylla* in China is further demonstrated by recent studies on its natural availability (10) and cultivation (11).

RESULTS AND DISCUSSION

Using standard phytochemical survey tests (12) a preliminary analysis of small samples of the drug suggested the possible presence of amino acids, saccharides or glycosides, phenols or tannins, flavonoids, coumarins, and sterols or triterpenes. Tests for saponins, alkaloids, organic acids, and anthraquinones were negative. This paper reports a more detailed examination of the lipid fraction.

Separation of the Et_2O- and hydrocarbon-soluble fractions by cc and preparative tlc led to the identification of palmitic and linoleic acids by comparison of their chromatographic and spectral properties with those of authentic samples. Also detected was a compound whose mass spectrum shows prominent peaks at *m/z* 134 and 74 as expected (13) for the McLafferty fragmentations of a 1-acylglycerol (Figure 1). This hypothesis was supported by the ^{13}C-nmr spectrum of this material, which shows a single ester carbonyl and three carbinol carbon atoms at δ 65.2, 70.3, and 63.4, close to the values (δ 66.4, 71.6, and 63.9) calculated by the "B.S." method (14) for C-1, C-2, and C-3 of a 1-acylglycerol. Further confirmation of this premise was available from an authentic sample of glycerol 1-monostearate, which also displays the above mass spectral features and ^{13}C spectrum (δ 65.0, 70.2, and 63.3). That the acyl group is un-

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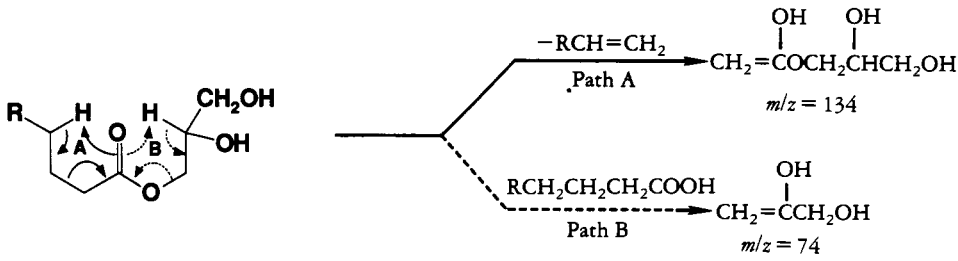

FIGURE 1. McLafferty fragmentations of a 1-acylglycerol.

saturated was demonstrated by the presence of at least five olefinic carbons. (Commercial linoleic acid shows the same five peaks, presumably due to the presence of structural or stereoisomers.) The remainder of the ^{13}C nmr shows at least 10 lines in the δ 14–35 region, one of which is twice and another three times the intensity of the other eight, consistent with a linolyl residue. The ^{1}H nmr is also in agreement with this assignment showing, in addition to the carbinol hydrogens of the glycerol residue, all the hydrogens found in the side chain of linoleic acid (cf. data for fraction A-1 in Experimental). Glycerol 1-monolinolate is a known compound (15) that is among the lipid components released from phorbol ester-stimulated cells (16).

The remaining, and most interesting, compound isolated from the lipid fraction was obtained in about 0.004% yield as an oil and displays surprisingly simple spectral properties. The ir spectrum shows a broad NH or OH peak centered at 3300 cm^{-1}, a strong conjugated carbonyl absorption at 1670 cm^{-1}, a pronounced out-of-plane aromatic CH bending mode at 745 cm^{-1}, and several other prominent vibrations in the fingerprint region. The ^{13}C nmr (Table 1) confirms the presence of a carbonyl group of an acid derivative (δ 161.0) as well as eight other sp^2 and one sp^3 carbon. The ^{1}H nmr (Table 1) displays a broad one-proton peak below δ 9 as expected for an NH or OH group, a two-proton singlet at δ 5.2 suggesting the attachment of two electron-withdrawing groups, and four one-proton and one two-proton multiplets in the aromatic region. Combining the above data, which indicate a partial molecular formula of $C_{10}H_9O$,

TABLE 1. Nmr Parameters for 3-Furfuryl Pyrrole-2-carboxylate.[a]

Position	Carbon	Hydrogen
1	—	9.38 (br, 1H)
2	122.6	—
3	115.6	6.95 (m, 1H)[b]
4	110.6	6.27 (m, 1H)
5	123.0	6.96 (m, 1H)[b]
6	161.0	—
2′	141.6	7.53 (m, 1H)
3′	120.6	—
4′	110.7	6.49 (m, 1H)
5′	143.4	7.41 (m, 1H)
6′	57.6	5.18 (s, 2H)

[a]COSY correlations indicated by brackets; CH correlations determined by HETCOR.

[b]These peaks overlap in the 1D spectrum but are clearly distinguished in the HETCOR experiment.

with the presence of a nitrogen atom as required by the odd molecular ion at *m/z* 191 leads to a molecular formula of $C_{10}H_9NO_3$.

The major structural features of this compound were elucidated with 2D nmr techniques (17). The normal HETCOR experiment leads to the C-H attachments shown in Table 1 and demonstrates that the two-proton peak at δ ca. 6.95 is in fact due to two methine groups while the one at δ 5.18 is due to a methylene group, a conclusion which was verified by an SFORD experiment. Although the proton multiplets are too complex for easy analysis, a COSY determination (Table 1) reveals two distinct spin systems, each containing three aromatic protons, as in two separate rings one of which has an attached methylene group. This analysis suggests two possible combinations: two furan rings connected by an amide group ($+CH_2$) or one furan and one pyrrole ring connected by an ester linkage ($+CH_2$).

The mass spectral fragmentation pattern (Figure 2) clearly points to the latter possibility with major ions at *m/z* 94 for pyrroyl (18) and 81 for furfuryl (19) but only minor ones for furoyl at 95 and pyrrylmethyl at 80. Only the position of substitution on the heterocyclic rings, therefore, remains to be determined. This was accomplished by a consideration of substituent effects on the proton and carbon chemical shifts of simple pyrroles (18) and furans (19), which suggested that the furan ring was β substituted, and the pyrrole ring was α substituted. The deduced structure, therefore, is 3-furfuryl pyrrole-2-carboxylate [**1**] which was confirmed by synthesis from 3-furfuryl alcohol and pyrrole-2-carboxylic acid via the sodium salt and acid chloride according to the procedure of Rapoport and Willson (20).

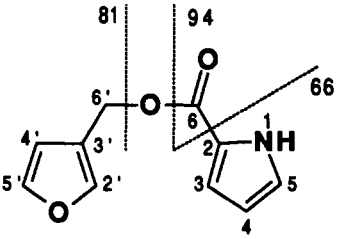

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FIGURE 2. Numbering and mass spectral fragmentations of **1**.

The complete assignment of the nmr parameters of **1** as shown in Table 1 requires distinguishing between the two α positions of the furan ring and between the two β positions of the pyrrole ring and also requires assigning the two quaternary carbon atoms. The first of these is based on the observed COSY correlation between H-6′ and H-2′ but not H-5′, while the remaining two were determined from the good agreement of calculated (18) and observed proton and carbon chemical shifts.

The isolation of simple aromatic esters such as **1** from plants is rare but not unknown; for example, a series of benzyl benzoates have been found in several species of *Aniba* (Lauraceae) (21). Although **1** is a new compound, both of its component parts are known in natural products. Pyrrole-2-carboxylic ester groups are found in the antibiotic milbemycin B (22), the alkaloids ryanodine (23) and calpurnine (24), and in the frog poison batrachotoxin (25) to cite just a few examples. 3-Furfuryl alcohol itself has been isolated from the essential oils of *Stellaria aquatica* (26) and *Aloe arborescens* (27), while its ester derivatives are found among the furano-diterpenes (28) and as the sponge metabolite pleraplysillin-2 (29).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were obtained on a Finnigan 1020 OWA

instrument at 70 eV with pertinent peaks above *m/z* 40 reported as *m/z* (rel. int.). Gc analyses were performed on the same instrument with a DB-1 30 m × 0.25 mm capillary column. Nmr spectra were taken on a Varian XL-300 instrument at 299.936 (^{1}H) and 75.427 (^{13}C) MHz in $DCCl_3$ solution with internal TMS standard at $\delta = 0$. Ir spectra were measured as thin films or KBr pellets on a Perkin-Elmer 237 instrument and are reported as ± 10 cm^{-1}. Preparative tlc plates were prepared in 1 mm thickness from Merck Si gel PF 254 + 366. Analytical tlc was performed on precoated sheets of Si gel 60 without indicator (EM Catalogue No. 5506) and developed with I_2 vapor. Cc was performed with 230–400 mesh Si gel 60 in glass columns unless otherwise noted.

ISOLATION.—The herbal drug tsai-zi-shen was purchased in Beijing in June 1984, and identified as the tuberous root of *P. heterophylla* by Mr. Cheng-yi Qian of the Beijing College of Traditional Chinese Medicine. This dried and ground drug (4 kg) was extracted by digestion with 1 × 2.0 and 3 × 1.2 liters of refluxing MeOH for 1 h. Evaporation of the MeOH left 526 g of a sticky mass which was mixed with 600 ml H_2O and extracted with 1 × 500 and 4 × 200 ml $CHCl_3$. The residue after removal of the $CHCl_3$ was separated into petroleum ether-soluble (A), Et_2O-soluble (B), and residue (C) fractions weighing 6.0, 4.4, and 3.2 g, respectively.

Fraction A was subjected to gradient elution cc on 400 g Si gel with hexane/EtOAc. A 9:1 mixture of these solvents eluted 800 mg of crystalline material which was further separated by preparative tlc [hexane-Me_2CO (6:4)] into two fractions. The faster-moving one (A-1) weighed 700 mg and consisted of a 35:65 mixture of palmitic and linoleic acids by gc-ms comparison of retention times and mass spectra ($[M]^+$ 256,280) with those of authentic samples. The presence of both acids was further confirmed by the ^{1}H nmr of A-1 which displayed, in approximately the ratio expected for this mixture, peaks at 5.35 (m, C*H*=C*H*), 2.78 (t, $J = 4.6$, CH=CHCH_2CH=CH), 2.35 (t, $J = 7.2$, CH_2C=O), 2.02 (m, CH$_2$CH_2CH=CH), 1.63 (m, CH_2CH$_2$C=O), 1.26 (m, other CH_2), and 0.88 (CH_3).

The slower-moving fraction (A-2) from the above tlc was dark under uv light and consisted of 19 mg of an oil which proved to be 3-furfuryl pyrrole-2-carboxylate by the identity of its spectral properties with those of a synthetic sample described below.

Fraction B plus 600 mg obtained in the same way from another extraction of 1 kg of drug (total 5.0 g), was subjected to dry cc on 200 g Si gel in a nylon tube (30) using $CHCl_3$-MeOH (9:1). After the solvent front reached the bottom of the column, the tube was sectioned. The fastest moving section consisted of 263 mg of a mixture which from tlc, gc-ms, ^{1}H-, and ^{13}C-nmr examination contained palmitic and linoleic acids and a contaminant of the common plasticizer di(2-ethylhexyl) phthalate. The second fastest moving section contained 1.87 g of a brown oil which was further separated by nine preparative tlc's with hexane-EtOAc (6:4) (2 developments). The two fastest moving bands contained 16 and 174 mg of A-1 and A-2, respectively, based on tlc and spectral comparisons. The slowest moving band (102 mg), which appeared yellow with I_2, was subjected to another preparative tlc with hexane-Me_2CO (6:4) to give 12 mg of a gum which appeared to be primarily glycerol 1-monolinolate from consideration of its spectral properties: ms *m/z* (%) $[M]^+$ 354 (0.2), 134 (19), 112 (12), 98 (49), 95 (20), 84 (29), 83 (28), 81 (29), 74 (31), 71 (20), 69 (41), 67 (41), 57 (65), 55 (93), 43 (100), 41 (82); ^{13}C nmr 174.3, 130.2, 130.0, 129.7, 128.0, 127.9, 70.3 (CH), 65.2, 63.4, 34.3, 32.1, 29.8 (× 3), 29.6, 29.5, 29.2 (× 2), 27.3, 25.1, 22.9, 14.3; ^{1}H nmr identical to those listed for fraction A-1 plus 4.17 (ddd, 2, CH$_2$OC=O), 3.93 (quin, 1, CHOH), and 3.65 (ddd, 2, CH$_2$OH).

3-FURFURYL PYRROLE-2-CARBOXYLATE.—A mixture of 1.11 g (10 mmol) of pyrrole-2-carboxylic acid and 5 ml H_2O was treated with 99 ml 0.1 N NaOH to pH 8.5 and the resulting solution evaporated to dryness at 105° and 2 mm Hg to leave 1.33 g (100%) of the sodium salt as a white powder. This was suspended in 25 ml dry C_6H_6, and 0.87 ml oxalyl chloride in 25 ml C_6H_6 was added and the resulting mixture heated at 50° for 45 min. A solution of 0.86 ml of 3-furfuryl alcohol and 0.8 ml dry pyridine in 15 ml C_6H_6 was added and the mixture allowed to stir for 4 h. The reaction mixture was washed with 3 × 50 ml 1 M HCl, 2 × 50 ml saturated $NaHCO_3$, and H_2O until the pH of the wash was 4–5. The organic layer was dried over $MgSO_4$ and evaporated to leave an oil which was chromatographed on 9 g of Si gel to give 467 mg (25%) of pure product and 382 mg (19%) which contained a trace of unreacted pyrrole acid. The tlc and gc retention behavior as well as the mass spectrum and ^{1}H- and ^{13}C-nmr parameters of the product were identical to those of the material in fraction A-2 above; ir (cm^{-1}) 3300 br, 1670 s, 1410 s, 1315 s, 1160 s, 1125 s, 1075, 1025, 960, 745 s; ms *m/z* (%) $[M]^+$ 191 (7), 94 (100), 81 (67), 66 (12), 53 (24); fragment structures see Figure 2; ^{13}C and ^{1}H nmr see Table 1.

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