Similarly, compounds **4**, **6**, **8**, and **10**, when treated with 0.25 equiv of $Pd(PPh_3)_4$ at room temperature for 24 h followed by acetylation, gave rise to **2b** (63%), **7b** (65%), **9b** (39%), and **11b** (62%), respectively. Yields were calculated on the basis of recovered starting material.

Preparation of 4β-(Benzylamino)withanolide D (13). To a solution of withanolide D 4-acetate (**6**; (76 mg, 0.148 mmol) and benzylamine (43.6 mg, 0.297 mmol; 2 equiv) in THF was added $Pd(PPh_3)_4$ (43.6 mg, 0.037 mmol). The mixture was stirred for 45 min under an N_2 atmosphere (until no starting material could be detected by TLC). The solvent was removed by an argon stream, and the residue so obtained was washed with ethyl acetate through a short bed of silica gel. Further purification by preparative TLC (eluted with hexane/ethyl acetate, 3:7) afforded **13** [R_f 0.75; yield 56.2 mg (58%)] as an amorphous powder. Physical data and spectral characteristics are given in Tables II–IV.

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Registry No. **1b**, 6128-32-1; **1c**, 20045-65-2; **2a**, 81644-34-0; **2b**, 81644-36-2; **4**, 79396-43-3; **5**, 22848-79-9; **6**, 22848-70-0; **7a**, 85151-00-4; **7b**, 85151-01-5; **8**, 74747-53-8; **9a**, 85151-02-6; **9b**, 85151-03-7; **10**, 71801-59-7; **11a**, 85151-04-8; **11b**, 85151-05-9; **12**, 122-57-6; **13**, 85151-06-0; benzylamine, 100-46-9; $Pd(PPh_3)_4$, 14221-01-3.

Supplementary Material Available: ^1H NMR traces for the reaction between $Pd(PPh)_3$ and compound **6** in C_6D_6 and in acidic C_6D_6; spin-saturation-transfer experiment between benzalacetone and its Pd complex (3 pages). Ordering information is given on any current masthead page.

Total Synthesis of (±)-Justicidin P. A New Lignan Lactone from *Justicia extensa*[1]

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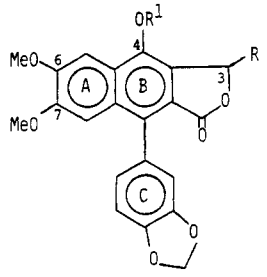
The total synthesis of (±)-justicidin P, a new lignan lactone from *Justicia extensa* (Acanthaceae), is described. Justicidin P exists at 25 °C as two rotamers, evidenced by proton NMR studies and confirmed by a theoretical calculation of the rotational barrier. Two other natural products, diphyllin and justicidin A, have also been synthesized.

In a continuing search for biologically active compounds from plant sources, Dr. N. G. Patel of our Department has studied the plant *Justicia extensa* (Acanthaceae), an African herbaceous plant obtained from Longwood Gardens, Kennett Square, PA. Extracts of its leaves show insecticidal and antiviral properties; the active principle, named justicidin P, was isolated and its structure determined as shown below on the basis of spectral data.[2] Justicidin P



1, R=OMe; R^1=Me (justicidin P)
2, R=H; R^1=Me (justicidin A)
3, R=R^1=H (diphyllin)

comprises about 1% of the dry leaves. Among hundreds of known natural lignans,[3] justicidin P is the first one which has a methoxy group on the lactone ring. Herein we describe a total synthesis of (±)-justicidin P, unambiguously confirming its structure.

Justicidin P is a C(3)-methoxylated derivative of justicidin A (**2**)[4] which can therefore be viewed as a synthetic precursor. Justicidin A, in turn, can be prepared from another natural lignan, diphyllin (**3**).[4a,5] Several syntheses of diphyllin and justicidin A have appeared in the literature.[6] A potentially convenient way to construct these arylnaphthalene lactones would involve conjugate addition of aryldithiane anion to 2-butenolide followed by alkylation with aryl aldehyde. In fact, several groups have successfully employed this methodology in the synthesis of various lignans.[7] We have thus synthesized diphyllin and justicidin A by this highly efficient route.[8]

With justicidin A in hand, the only remaining step would be introduction of the C(3) methoxy group. This was

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(2) The isolation and structure determination of justicidin P will be published elsewhere.

(3) D. C. Ayres, "Chemistry of Lignans", Andhra University Press, India, 1978.

(4) (a) M. Okigawa, T. Maeda, and N. Kawano, *Tetrahedron*, **26**, 4301 (1970); (b) K. Ohta, S. Marumo, Y.-L. Chen, and K. Munakata, *Agric. Biol. Chem.*, **35**, 431 (1971).

(5) A. G. Conzalez, V. Darias, and G. Alonso, *Planta Med.*, **36**, 200 (1979), and references cited therein.

(6) (a) H. P. Plaumann, J. G. Smith, and R. Rodrigo, *J. Chem. Soc., Chem. Commun.*, 354 (1980). (b) Z.-I. Horii, K. Ohkawa, S.-W. Kim, and T. Momose, *Chem. Pharm. Bull*, **19**, 535 (1971); *J. Chem. Soc., Chem. Commun.*, 653 (1968). For other syntheses of lignans see ref 3 and (c) T. Biftu, B. G. Hazr, and R. Stevenson, *J. Chem. Soc., Perkin Trans. 1*, 2276 (1979); (d) P. A. Ganeshpure and R. Stevenson, *ibid.*, 1681 (1981); (e) E. Brown, M. Loriot, and J.-P. Robin, *Tetrahedron Lett.*, 1389 (1979); (f) P. A. Ganeshpure, G. E. Schneiders and R. Stevenson, *ibid.*, **22**, 393 (1981); (g) A. I. Meyers and W. B. Avila, *J. Org. Chem.*, **46**, 3881 (1981); (h) J. Mann and S. E. Piper, *J. Chem. Soc., Chem. Commun.*, 430 (1982); (i) E. Brown, M. Loriot, and J.-P. Robin, *Tetrahedron Lett.*, **23**, 949 (1982).

(7) (a) F. E. Ziegler and J. A. Schwartz, *Tetrahedron Lett.*, 4643 (1975); (b) Y. Asano, T. Kamikawa, and T. Tokoroyama, *Bull. Chem. Soc. Jpn.*, **49**, 3232 (1976); (c) R. E. Damon, R. H. Schlessinger, and J. F. Blount, *J. Org. Chem.*, **41**, 3772 (1976); (d) F. E. Ziegler and J. A. Schwartz, *ibid*, **43**, 985 (1978); (e) A. G. Gonzalez, J. P. Perez, and J. M. Trujillo, *Tetrahedron*, **34**, 1011 (1978); (f) G. B. Mpango and V. Snieckus, *Tetrahedron Lett.*, **21**, 4827 (1980); (g) A. Pelter, P. Satyanarayana, and R. S. Ward, *ibid.*, **22**, 1549 (1981); (h) A. Pelter, R. S. Ward, P. Satyanarayana, and P. Collins, *ibid.*, **23**, 571 (1982).

(8) Justicidin A was prepared from 2-(3,4-dimethoxyphenyl)-1,3-dithiane in ca. 30% overall yield by the following transformations: (1) (a) *n*-BuLi, (b) 2-butenolide, (c) piperonal; (2) CF_3CO_2H; (3) HgO/BF_3–Et_2O; (4) $PyH^+Br_3^-/AcOH$;[9] (5) MeI/K_2CO_3.

(9) For aromatization of the B ring that gives diphyllin.

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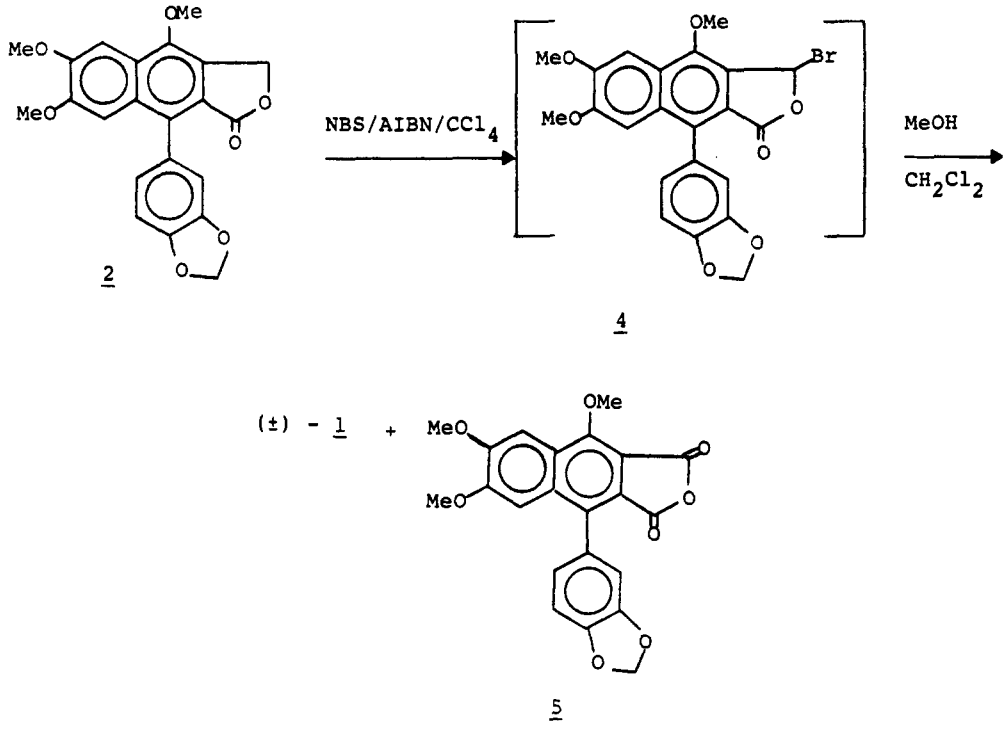
Scheme I

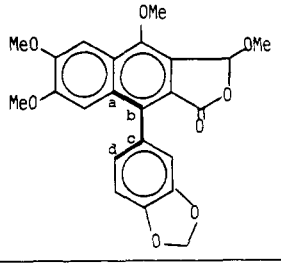


accomplished by benzylic bromination with *N*-bromosuccinimide using benzoyl peroxide as radical initiator followed by treatment of the crude bromide **4** with methanol to afford (±)-justicidin P in 25% yield (Scheme I). The major side product (25%) was anhydride **5**. Fortunately, when benzoyl peroxide was replaced by azobis[isobutyronitrile] (AIBN), (±)-**1** could be isolated in 71% yield without any **5** detected. It is interesting that the bromide in **4** can be readily replaced by a hydroxyl group from moisture in the air. This unusual reactivity is probably due to the double activation by two methoxy groups (C_4 and C_7). Synthetic (±)-justicidin P is identical in TLC mobility in several solvent systems and in spectral properties (NMR, IR, UV, and Fluorescence) with natural justicidin P.

It is worthwhile to mention some NMR studies of justicidin P. The proton NMR spectrum of justicidin P shows two singlets in $CDCl_3$ at δ 5.97 which correspond to the methylenedioxy protons. In toluene-d_8, these two singlets become two triplets (resulting from two AB quartets). When the temperature is raised to 80 °C, these two triplets gradually collapse to two broad singlets. Finally, at 120 °C, they become a sharp singlet. Cooling to room temperature reestablishes the two triplets. These studies clearly demonstrate that at 25 °C justicidin P exists as two rotamers. The relative energies of the various conformations of justicidin P were calculated by using Allinger's molecular mechanics (MMPI) program[10] at fixed $C_aC_bC_cC_d$ torsion angles. The results are shown in Table I. As expected, the energy minima occur when the rings are approximately perpendicular to each other and the energy maxima when they are planar. The barrier appears to be about 18–19 kcal/mol which is large enough to show rotational isomers in the NMR at room temperature.

In summary, we have confirmed the structure of justicidin P by a short and efficient total synthesis which consists of seven steps in ca. 20% yield. Justicidin P exists at 25 °C as two rotamers. In addition, two other natural products, diphyllin and justicidin A, have also been synthesized.

Table I



dihedral angle (a,b,c,d), deg	ΔE (above min), kcal/mol	dihedral angle (a,b,c,d), deg	ΔE (above min), kcal/mol
0	18.55	180	18.83
60	0.80	240	0
90	1.16	270	1.53
120	0.55	300	0.52

Experimental Section

Melting points were determined on a Laboratory Devices Mel-Temp melting point apparatus and are uncorrected. IR spectra were obtained by using a Perkin-Elmer 137 sodium chloride or a Beckman AccuLab 8 spectrophotometer. NMR spectra were determined with an HR-200 or an EM-390 spectrometer with tetramethylsilane as the internal reference. Mass spectra were recorded on a Vg-MM-7070H high-resolution mass spectrometer.

(±)-9-(1,3-Benzodioxol-5-yl)-3,4,6,7-tetramethoxynaphtho[2,3-*c*]furan-1(3*H*)-one (1, (±)-Justicidin P). A mixture of **2** (1.25 g, 3.17 mmol) in carbon tetrachloride (150 mL) containing *N*-bromosuccinimide (recrystallized, 0.68 g, 3.8 mmol) and AIBN (100 mg) was heated at ca. 85° for 3 h. After the solvent was removed, the residue (crude **4**) was dissolved in methanol–methylene chloride (20 mL each), and the reaction was stirred for 3 h at room temperature. Removal of the solvent and purification by HPLC provided 950 mg (71%) of (±)-**1** as a white solid: mp 208–210 °C (202 °C dec); IR (CH_2Cl_2) 1740 cm^{-1}; NMR ($CDCl_3$) δ 7.48 (s, 1 H), 6.92 (s, 1 H), 6.86–6.66 (m, 3 H), 6.44 (s,

(10) N. L. Allinger, J. A. Hirsch, M. A. Miller, I. J. Tyminski, and F. A. Van-Catledge, *J. Am. Chem. Soc.*, **90**, 1199 (1968).

1 H), 5.97 (2 s, 2 H), 4.16 (s, 3 H), 4.00 (s, 3 H), 3.72 (s, 3 H), 3.59 (s, 3 H); UV (EtOH) 264, 295, 309, 350 nm; fluorescence 453 nm; HRMS, m/z 424.1151 (M^+), calcd for $C_{23}H_{20}O_8$ 424.1155.

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Registry No. 1, 86012-93-3; 2, 25001-57-4; 4, 86012-94-4; 5, 86012-95-5.

Chemical Synthesis of Some Mono- and Digalactosyl *O*-Glycopeptides†

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Chemical syntheses of several *O*-glycopeptides containing *O*-galactosylthreonine are reported. Two different approaches have been investigated to determine the strategy best adapted to the synthesis of any desired *O*-glycopeptide. Glycosylation of an adequately protected threonine-containing peptide was shown to be less successful than a stepwise strategy using an appropriate *O*-glycosyl amino acid as starting material. This route was shown to have more potential since it allows construction of complex glycopeptides containing both glycosyl amino acids and unglycosylated hydroxy amino acids. Various α- and β-*O*-glycopeptides are described in which the threonine molecule linked to galactose is either C-terminal or N-terminal or inserted in the peptide chain.

We have recently reported the one-step synthesis of *O*-glycosyl amino acids relevant to glycoproteins.[1] The procedure involved the reaction of a fully benzylated reducing sugar with the appropriate derivative of a serine, threonine, or hydroxyproline molecule, in the presence of trifluoromethanesulfonic anhydride, followed by removal of all protecting groups by hydrogenolysis in the presence of 10% palladium-on-charcoal as catalyst. We now wish to report the synthesis of several threonine-containing *O*-glycopeptides of known anomeric configuration in which the threonine molecule linked to galactose is either C- or N-terminal or inserted in the peptide core.

In the past few years, natural abundance carbon-13 nuclear magnetic resonance has been used to gain dynamic and structural information about carbohydrate residues of large glycoproteins.[2-4] Application of this technique to the structural study of glycoproteins seems promising, but there are still some temporary limitations. One of these is the lack of ^{13}C NMR data of relevant model compounds needed to make specific assignments in the spectra of glycoproteins. Knowledge of ^{13}C NMR chemical shifts of appropriate model compounds may facilitate the use of this technique to study carbohydrate–peptide linkages in intact glycoproteins.

In addition, the glycopeptides reported in this communication were required in the course of our research to provide a better understanding of the structural and conformational properties of the glycosidic bond in glycopeptides as well as the role of the carbohydrate moiety in the binding of metal cation with membrane glycoproteins.

Results and Discussion

One approach to the synthesis of glycopeptides is the synthesis of the core peptide containing the desired hydroxy amino acid followed by glycosylation. To be suitable, this approach requires the glycosylation method to be highly stereoselective. Moreover, since the glycosylation reaction depends upon the reactivity of the hydroxyl group, this reactivity should not be altered by the incorporation of serine, threonine, or hydroxyproline in the peptide chain. In fact, as seen below, this latter requirement was not achieved in all cases. An alternative approach requires the synthesis of adequately protected *O*-glycosyl amino acids, followed by elongation at either or both the C- or N-terminals. As done previously,[5-8] we followed the latter strategy. This route has more potential since it allows construction of complex glycopeptides containing both glycosyl amino acids and unglycosylated hydroxy amino acids. It should be noted that Guillemin and co-workers[9] attempted to adapt this route to the solid-phase synthesis of *N*-glycopeptides related to somatostatin.

In order to determine the strategy best adapted to the synthesis of any desired glycopeptide, both of these routes were investigated. Peptide blocking strategies are well-known. However, in the case of glycopeptides, there are additional requirements: (i) Deprotection of peptide residues to allow coupling at the C- and N-terminal must be both selective and compatible with carbohydrate hydroxyl-protecting groups. (ii) The preparation of glycopeptides with the α-anomeric configuration precludes the presence of participating neighboring groups on the carbohydrate moiety. Therefore, benzyl derivatives were preferred to acetyl or benzoyl derivatives. (iii) Amino acid protecting groups, on the other hand, must be stable in acid medium because of the acid-catalyzed nature of the glycosylation reaction.[10] Ideally, the removal of glycosyl

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(1) J. M. Lacombe, A. A. Pavia, and J. M. Rocheville, *Can. J. Chem.*, **59**, 482-489 (1981).
(2) K. Dill and A. Allerhand, *J. Biol. Chem.*, **254**, 4524-4531 (1979).
(3) E. Berman, A. Allerhand, and A. L. DeVries, *J. Biol. Chem.*, **255**, 4407-4410 (1980).
(4) E. Berman, D. E. Walters, and A. Allerhand, *J. Biol. Chem.*, **256**, 3853-3857 (1981).
(5) J. Martinez, A. A. Pavia, and F. Winternitz, *Carbohydr. Res.*, **50**, 15-22, 148-151 (1976).
(6) H. G. Gard and R. W. Jeanloz, *Carbohydr. Res.*, **52**, 245-250 (1976).
(7) H. G. Garg, R. W. Jeanloz, *Pept. Proc. Am. Pept. Symp. 5th*, 477-479 (1977); *Chem. Abstr.*, **89**, 454489 (1978).
(8) M. G. Vafina and W. A. Derevitzkaya, *Izv. Akad. Nauk SSSR, Ser. Khim.* 1475 (1965).
(9) S. Lavielle, N. Ling, R. Saltman, and T. Guillemin, *Carbohydr. Res.*, **89**, 229-236 (1981).

0022-3263/83/1948-2557$01.50/0