JOURNAL OF NATURAL PRODUCTS

Subscriber access provided by ISTANBUL TEKNIK UNIV

2-Hydroxymethyl-3,4-dihydroxy-6-methylpyrrolidine (6-Deoxy-DMDP), an Alkaloid #-Mannosidase Inhibitor from Seeds of Angylocalyx pynaertii

Russell J. Molyneux, Y. T. Pan, Joseph E. Tropea, Alan D. Elbein, Carl H. Lawyer, David J. Hughes, and George W. J. Fleet

J. Nat. Prod., **1993**, 56 (8), 1356-1364• DOI: 10.1021/np50098a020 • Publication Date (Web): 01 July 2004

Downloaded from http://pubs.acs.org on April 4, 2009

More About This Article

The permalink http://dx.doi.org/10.1021/np50098a020 provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

ACS Publications
High quality. High impact.

Journal of Natural Products is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Journal of Natural Products
Vol. 56, No. 8, pp. 1356-1364, August 1993

2-HYDROXYMETHYL-3,4-DIHYDROXY-6-METHYLPYRROLIDINE (6-DEOXY-DMDP), AN ALKALOID β-MANNOSIDASE INHIBITOR FROM SEEDS OF *ANGYLOCALYX PYNAERTII*

RUSSELL J. MOLYNEUX,*

Western Regional Research Center, Agricultural Research Service, USDA, Albany, California 94710

Y.T. PAN, JOSEPH E. TROPEA,[1] ALAN D. ELBEIN,

Department of Biochemistry and Molecular Biology, School of Medicine, University of Arkansas, Little Rock, Arkansas 72205

CARL H. LAWYER,

Lawyer Nursery, Plains, Montana 59859

DAVID J. HUGHES, and GEORGE W.J. FLEET

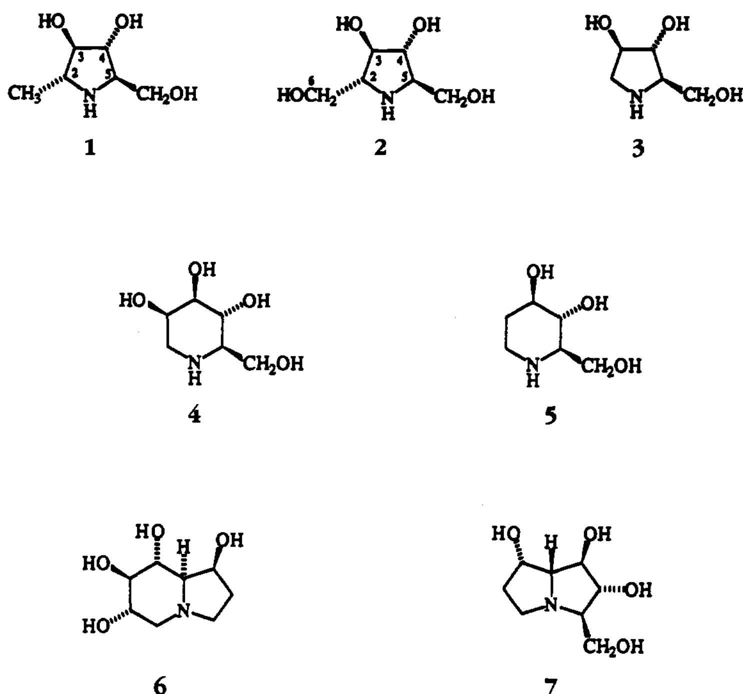
Dyson Perrins Laboratory, University of Oxford, Oxford OX1 3QY, UK

ABSTRACT.—A polyhydroxy alkaloid has been isolated from the seeds of the African legume *Angylocalyx pynaertii* and identified as a 2-hydroxymethyl-3,4-dihydroxy-5-methylpyrrolidine by ms and ^{1}H- and ^{13}C-nmr spectroscopy. The absolute stereochemistry was established, by a stereochemically unambiguous synthesis from diacetone glucose, as 2,5-imino-1,2,5-trideoxy-D-mannitol, which may also be regarded as 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) [**2**] in which a hydroxymethyl group is deoxygenated, i.e., 6-deoxy-DMDP [**1**]. Whereas the structurally related polyhydroxypyrrolidine alkaloids which have previously been discovered are inhibitors of α- and β-glucosidase, 6-deoxy-DMDP is unique in inhibiting β-mannosidase. In addition to this novel alkaloid and 2-hydroxymethyl-3,4-dihydroxypyrrolidine [**3**], previously shown to be present in several *Angylocalyx* species, the known piperidine alkaloids deoxymannojirimycin [**4**] and fagomine [**5**] were identified for the first time as constituents of *An. pynaertii* seeds.

Over the past decade, an increasing number of structurally related monocyclic and bicyclic polyhydroxy alkaloids have been isolated. The search for such compounds has been stimulated by their general property of glycosidase inhibition and potential utility as biochemical tools for the study of metabolic disorders caused by incomplete cellular processing of glycoproteins (1). Although the alkaloids have been found in both plants and microorganisms, a particularly fruitful source has been the plant family Leguminosae. Many of these alkaloids and structurally related analogues have been synthesized and their glycosidase inhibitory properties evaluated, in an attempt to determine the relationship of structure and stereochemistry to the observed biological properties (2).

The legume group *Angylocalyx* (tribe Sophoreae) encompasses three genera, namely *Castanospermum, Angylocalyx,* and *Xanthocercis* (3). The major alkaloidal constituent of *Castanospermum australe*, the Moreton Bay chestnut or black bean, is the polyhydroxy indolizidine castanospermine [**6**] (4), which has been the subject of considerable interest due to its potent α- and β-glucosidase inhibitory properties (5,6) and consequent antiviral, antihyperglycemic, and insect antifeedant properties (7–12). A number of additional bicyclic polyhydroxy alkaloids have been isolated from *C. australe*, including several epimers and a deoxy derivative of castanospermine (13–15), together with the pyrrolizidine alkaloid australine [**7**] and three epimers thereof (16–18). In contrast, *Xanthocercis* and *Angylocalyx* species have yielded only monocyclic polyhydroxy alka-

[1]NIDDK, National Institutes of Health, Bethesda, MD 20892.



loids. The 4-*O*-(β-D-glucosyl) derivative of fagomine [**5**], a piperidine alkaloid, has been isolated from *Xanthocercis zambesiaca* but was not found to possess any glycosidase inhibitory properties (19). The genus *Angylocalyx* consists of seven species of shrubs and trees growing in tropical African forests (3). Six of these species have been examined, and 2-hydroxymethyl-3,4-dihydroxypyrrolidine [**3**] has been isolated from the seeds and fruits of *Angylocalyx boutiqueanus* and shown by paper ionophoresis to be present in the other five species investigated, including *Angylocalyx pynaertii* DeWild. (20).

The availability of a small collection of seeds and pods of *An. pynaertii*, from the border of the Republic of Cameroon and the Central African Republic, provided an opportunity to examine the total alkaloid extract in detail, in order to determine whether bicyclic polyhydroxy alkaloids similar in structure to castanospermine or australine might be present. Although none were detected, four monocyclic pyrrolidine and piperidine alkaloids were identified as constitutents of the seeds. These included 2-hydroxymethyl-3,4-dihydroxypyrrolidine [**3**], deoxymannojirimycin [**4**], and fagomine [**5**]. In addition, a new polyhydroxypyrrolidine alkaloid, which was shown to be a competitive inhibitor of β-mannosidase, was isolated. The structure of the latter has now been established as 2-hydroxymethyl-3,4-dihydroxy-5-methylpyrrolidine (i.e., 6-deoxy-2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine; 6-deoxy-DMDP) by the application of 1D and 2D high-field nmr techniques and by ms. The absolute configuration was established by stereospecific synthesis from diacetone glucose as that of 2,5-imino-1,2,5-trideoxy-D-mannitol [**1**].

RESULTS AND DISCUSSION

Extraction of the ground seeds of *An. pynaertii* with MeOH, followed by ion-exchange chromatography on Dowex 50W-X8, gave the total alkaloid fraction as a pale brown viscous oil. Preliminary screening of this crude extract against a series of glycosidases showed that the extract had significant inhibitory activity towards α- and β-mannosidase and α-glucosidase. Analytical tlc showed the presence of three or more

constituents similar in mobility to castanospermine, but on spraying the plate with Ac_2O/Ehrlich's reagent these compounds failed to give the purple or pink coloration typical of polyhydroxy-indolizidine or -pyrrolizidine alkaloids, respectively (21). Instead, a yellow-brown color was produced, and a similar color was generated on treatment with ninhydrin, whereas no reaction occurred with Dragendorff's reagent. All of these color reactions are characteristic of polyhydroxy-pyrrolidine and -piperidine alkaloids.

Further examination of the per-trimethylsilylated alkaloid extract by capillary gc-ms showed the presence of five components with retention times of 11.36 (8%), 11.70 (43%), 12.17 (6%), 12.47 (3%), and 14.29 (36%) min. The major component (43%), eluting at 11.70 min, had an ms consistent with that expected for the tetra-TMSi derivative of 2-hydroxymethyl-3,4-dihydroxypyrrolidine, previously isolated from *An. boutiqueanus* (20) as well as from the fern *Arachniodes standishii* (22), the absolute configuration of which has been established as 2*R*,3*R*,4*R* (i.e., 1,4-dideoxy-1,4-imino-D-arabinitol [**3**]), by synthesis (23,24). In particular, the ms showed a molecular ion at *m/z* 421 and a base peak at *m/z* 318, corresponding to loss of a -CH_2OTMSi fragment. Although the identity of the compound in *An. pynaertii* was established only by gc-ms, biosynthetic considerations suggest that it probably has the same absolute configuration as that isolated from *An. boutiqueanus*. The alkaloid was found to be a potent inhibitor of amyloglucosidase and therefore accounts, at least in part, for the α-glucosidase inhibitory activity of the *An. pynaertii* extract.

The second most abundant alkaloid present, 36% of the total extract, had a retention time of 14.29 min, indicative of a more highly hydroxylated compound. The ms was consistent with that of the penta-TMSi derivative of deoxymannojirimycin [**4**] or its 5-epimer, deoxynojirimycin, showing a molecular ion at *m/z* 523 and a base peak at *m/z* 420, again due to loss of the -CH_2OTMSi moiety. Authentic samples of deoxynojirimycin and deoxymannojirimycin TMSi derivatives gave ms that were virtually identical, except for a slightly enhanced relative abundance of the fragment ion at *m/z* 330 in the case of the latter. However, the gc retention times were distinctly different, with deoxynojirimycin eluting at 14.91 min and deoxymannojirimycin co-eluting with the *An. pynaertii* alkaloid at 14.29 min. The second alkaloid, which also showed the more intense *m/z* 330 ion, was therefore established as deoxymannojirimycin [**4**]. This is the first reported occurrence of this piperidine alkaloid in a legume since its initial isolation from seeds of *Lonchocarpus sericeus* (25). Deoxymannojirimycin is a potent inhibitor of α-fucosidase and a moderate inhibitor of α-mannosidase, potentially contributing to the inhibitory activity of the crude alkaloid extract toward the latter enzyme.

In a similar manner, by correlation of gc retention time and identity of ms, a third component eluting at 12.47 min was identified as the piperidine alkaloid fagomine [**5**], comprising only 3% of the total alkaloid mixture. This alkaloid, which is structurally related to deoxymannojirimycin [**4**] but lacks the 5-OH group, was first isolated from buckwheat *(Fagopyrum esculentum)* (26) and has also been isolated from *Castanospermum australe* (16). The constituent eluting most rapidly on gc analysis (11.36 min), accounting for 8% of the extract, gave an ms with a molecular ion at *m/z* 363 and fragmentation pattern atypical of that observed for any of the bicyclic alkaloids, or for pyrrolidine and piperidine alkaloids which bear an exocyclic -CH_2OH group. On the basis of an identical gc retention time and the close similarity of its ms to that of an authentic sample, this component of the extract was identified as *N*-methyl-β-hydroxynorvaline.

The three known alkaloids identified in the *An. pynaertii* extract accounted for the inhibitory activity towards α-mannosidase and α-glucosidase but failed to account for its inhibition of β-mannosidase. Only a single component, eluting at 12.17 min, remained to be identified, and it appeared probable that the activity resided in this compound, which comprised 6% of the mixture. The gc-ms, which was remarkably

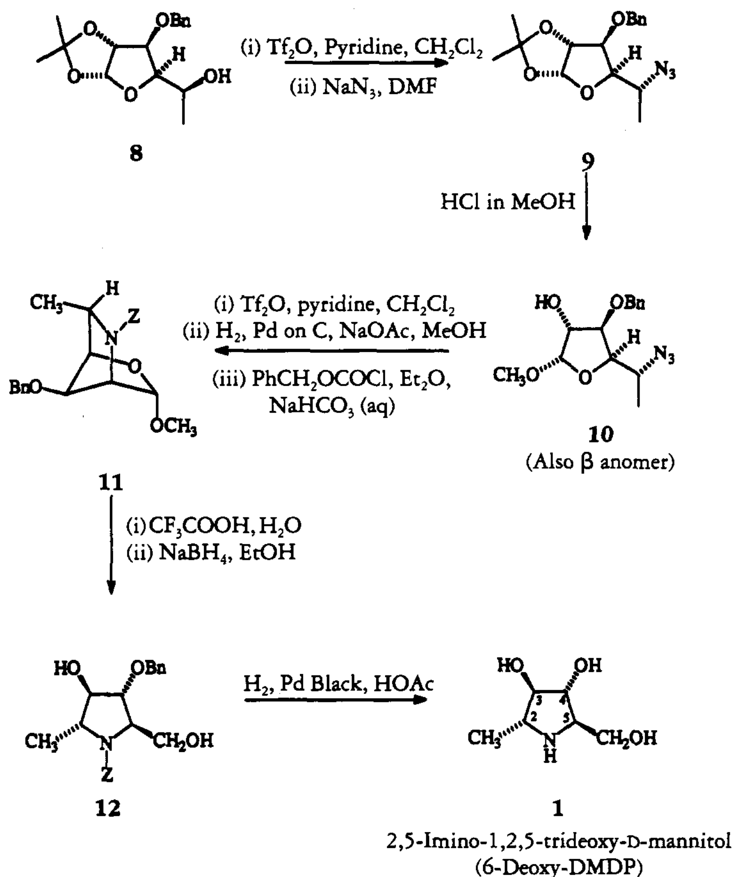
similar to that of the tetra-TMSi derivative of fagomine [**5**], showed a molecular ion at *m/z* 435 and a base peak at *m/z* 332 corresponding to loss of a -CH_2OTMSi fragment. It therefore seemed most likely that the alkaloid was an epimer of fagomine, but it was essential to isolate this particular compound in order to determine its structure and stereochemistry as well as to establish that it was the potential β-mannosidase inhibitor.

The crude alkaloid extract was refractionated on a Dowex 50 column with gradient elution, and representative fractions were monitored for inhibitory activity against α-glucosidase and α- and β-mannosidase, yielding three distinct bands of activity. The first band to elute showed inhibitory activity only towards α-mannosidase and was shown by gc-ms of its TMSi derivative to be identical in retention time and fragmentation pattern with deoxymannojirimycin [**4**]. The nmr spectrum was also consistent with that of the latter alkaloid, as was the optical rotation. The third band to elute was identified by gc-ms as a mixture consisting primarily of 2-hydroxymethyl-3,4-dihydroxypyrrolidine [**3**] and fagomine [**5**]. The second band, however, which exhibited significant inhibition of β-mannosidase, appeared to consist primarily of the novel alkaloid. Further purification by centrifugal radial tlc gave the alkaloid as a pale yellow oil which showed a single peak at 12.17 min on gc-ms of its TMSi derivative.

The molecular formula of the alkaloid was determined by hrms to be $C_6H_{13}NO_3$ with the base peak at *m/z* 116 corresponding to loss of a -CH_2OH moiety from the molecular ion. An analogous fragmentation was observed on gc-ms of the TMSi derivative. The compound was, therefore, a piperidine alkaloid isomeric with fagomine [**5**], or a pyrrolidine alkaloid having two exocyclic carbon atoms, at least one of which is the hydroxymethyl functionality.

The ^{13}C nmr established unequivocally that the alkaloid had a pyrrolidine ring system, since in addition to the -CH_2OH group signal at δ 65.1 it exhibited a peak at δ 19.9 consistent with the presence of an Me group. The remaining four signals corresponded to methine carbon atoms bearing OH groups or adjacent to the heterocyclic nitrogen atom. The gross structure of the alkaloid was therefore established as 2-hydroxymethyl-3,4-dihydroxy-5-methylpyrrolidine. The high-resolution ^{1}H-nmr spectrum supported this distribution of substitutents around the pyrrolidine ring, a ^{1}H-^{1}H homonuclear shift correlation experiment confirming the assignment of chemical shifts. The Me group was observed as a doublet with a coupling constant of 6.7 Hz. The pyrrolidine ring protons had coupling constants of 8.3, 7.0, and 7.0 Hz, consistent in value with those of 7.7, 7.1, and 7.7 Hz reported for the all-trans configuration of 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) [**2**], first isolated from *Derris elliptica* (27). These data suggested that the alkaloid was 6-deoxy-DMDP [**1**], but the well-known sensitivity of J values to slight variations in θ in five-membered ring systems rendered the suspected trans-, trans-, trans- arrangement of the ring protons somewhat equivocal. Unfortunately, since the compound was obtained as an oil, and attempts to form a crystalline HCl salt were unsuccessful, X-ray crystallography was not a viable option for determination of the configuration.

In order to establish the relative and absolute stereochemistries of the natural product, several stereoisomers of 6-deoxy-DMDP were synthesized. Complete details of the synthesis of these isomers and their glycosidase inhibitory properties will be the subject of a separate publication. The identity of 6-deoxy-DMDP was firmly established by a stereochemically unambiguous synthesis (Scheme 1), in which the ring nitrogen was introduced with overall retention of configuration at C-5 and with inversion of configuration at C-2 of D-glucose. Diacetone glucose was converted to the protected 6-deoxy-L-iditol [**8**] (28) which on reaction with triflic anhydride and subsequent treatment with sodium azide gave the gluco-azide [**9**] (29). Methanolysis of the acetonide in **9** gave a mixture of methyl furanosides. The α-furanoside **10** was reacted



SCHEME 1

with triflic anhydride to give the corresponding azidotriflate; hydrogenation of the azide in MeOH in the presence of palladium and NaOAc gave an amine which spontaneously cyclized to afford, after protection of the nitrogen by the benzyloxycarbonyl moiety, the bicyclic pyrrolidine **11**. Subsequent hydrolysis of the methyl furanoside with aqueous trifluoracetic acid and borohydride reduction of the resulting lactol gave **12**, from which the Z- and benzyl protecting groups were removed by hydrogenolysis to give 6-deoxy-DMDP with ^{13}C- and ^{1}H-nmr spectra identical to those of the natural product. Both the natural and synthetic compounds gave the same retention times and fragmentation patterns on gc-ms of the TMSi derivatives. Co-injection gave a single peak at 12.17 min. The correspondence in optical rotation values showed that the alkaloid had the same absolute configuration as the synthetic material, establishing the identity of 6-deoxy-DMDP as 2,5-imino-1,2,5-trideoxy-D-mannitol [**1**].

The glycosidase inhibition assays used to direct the isolation of 6-deoxy-DMDP had indicated that the alkaloid was an inhibitor of β-mannosidase but not of α-mannosidase or α-glucosidase. The inhibitory properties of the pure compound were therefore evaluated against a series of hydrolytic enzymes. 6-Deoxy-DMDP was a reasonably good competitive inhibitor of β-mannosidase, requiring a concentration of 380 μM to achieve 50% inhibition of enzymatic actvity (Figure 1). α-Fucosidase and β-galactosidase were inhibited at high concentrations, but no inhibition of α-mannosidase, α-galactosidase, α- or β-glucosidase, and β-xylosidase was observed. The absence of inhibition of either α- or β-glucosidase is somewhat unexpected in view of the structural similarities to

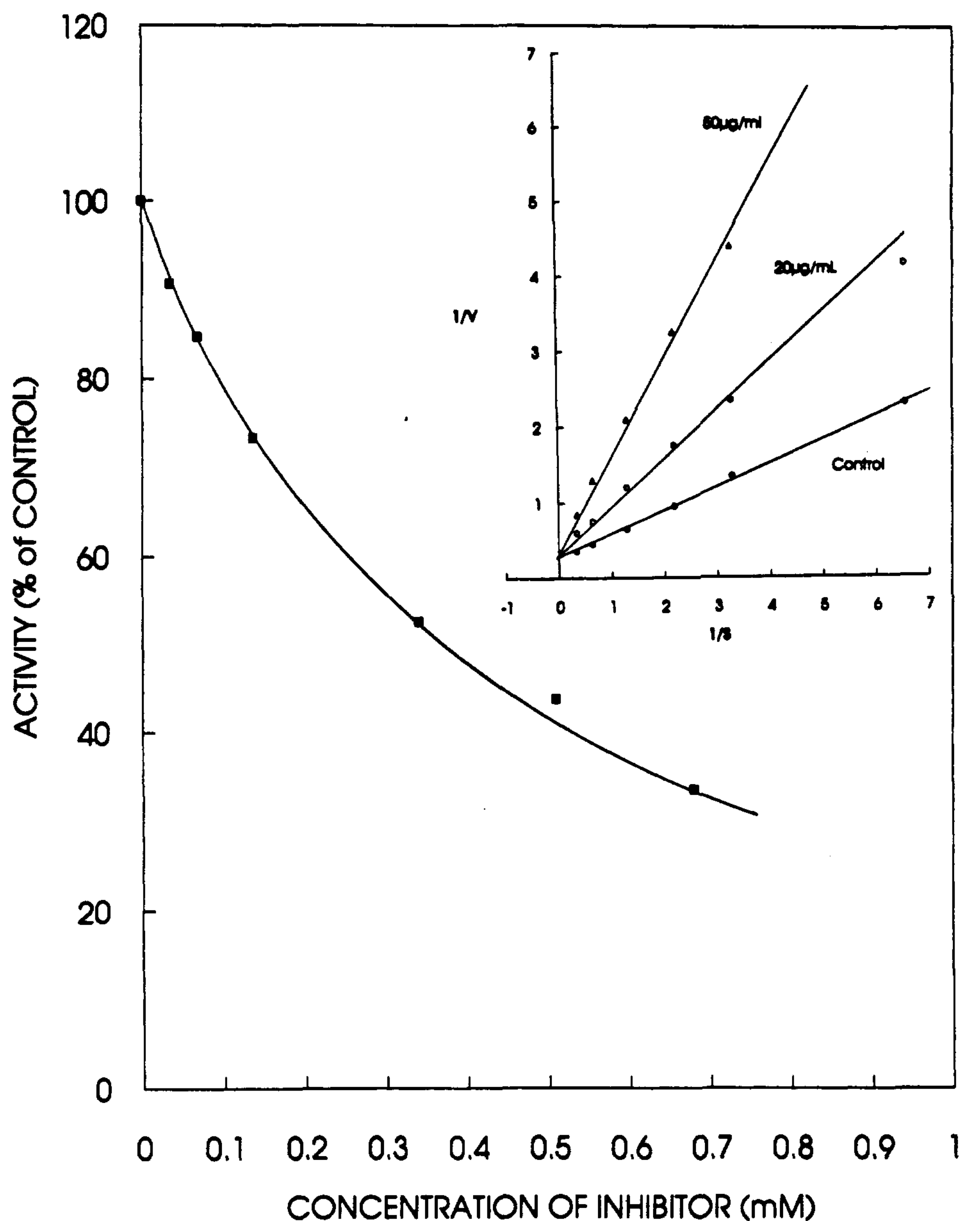


FIGURE 1. Inhibition of β-mannosidase by 6-deoxy-DMDP [1]. Activity is expressed relative to untreated enzyme. Inset shows kinetic analysis of 6-deoxy-DMDP against β-mannosidase according to the method of Lineweaver and Burk.

DMDP [2] and 2-hydroxymethyl-3,4-dihydroxypyrrolidine [3], which are both good inhibitors of these enzymes. A number of alkaloids, including deoxymannojirimycin [4], swainsonine, and several synthetic analogues, are potent inhibitors of α-mannosidase. In contrast, 6-deoxy-DMDP is the first to be isolated which inhibits β-mannosidase. It has been suggested that the structural requirements for inhibition of β-D-mannosidase are more rigorous than those for α-D-mannosidase (30). The unique inhibitory properties of 6-deoxy-DMDP may provide significant information regarding the arrangement and configuration of OH substituents necessary for inhibition of β-mannosidase, in addition

to indicating possible synthetic polyhydroxy alkaloid analogues specifically designed to inhibit this enzyme.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The alkaloids were monitored for purity by tlc on 0.25 mm Si gel plates developed with $CHCl_3$-MeOH-NH_4OH-H_2O (70:26:2:2) and detected by spraying with Ac_2O followed by Ehrlich's reagent (21). Homogeneity was also checked by gc-ms of the TMSi derivatives, prepared by treatment with MSTFA in pyridine, on a Hewlett-Packard 5890 Series II instrument equipped with a 5971 mass-selective detector operating at 70 eV, on-column injector, and a 60-m×0.32-mm i.d. SE-30 fused Si column. The column was temperature-programmed from 120° to 300° at 10°/min. Low resolution ei and ci (NH_3) ms were obtained on a VG Micromass 7070 mass spectrometer; hrms measurements were determined on the same instrument. Optical rotations were measured in a 1-dm cell on a Perkin-Elmer 241 automatic polarimeter. Nmr spectra were determined in D_2O on a Nicolet NTC 200FT spectrometer, with software package, at 200 MHz (^1H) and at 50.3 MHz (^{13}C) using 3-(trimethylsilyl)-1-propanesulfonic acid (TSP) as an internal standard. Multiplicities for ^{13}C signals were determined by application of the carbon attached proton test (CAPT) sequence and chemical shifts were assigned by a 2D heteronuclear ^1H-^{13}C shift correlation experiment. Preparative centrifugal tlc was performed on a Chromatotron Model 7924 (Harrison Research, Palo Alto, CA).

PLANT MATERIAL.—Seeds of *An. pynaertii* were collected on 8 June 1988 by J. Michael Fay and David J. Harris, in the Republic of Cameroon (02°21′N, 16°08′E, altitude 385 m), on the border with the Central African Republic, Sangha Economique Prefecture, across the river from Ndakan Gorilla Study Area. A voucher specimen (No. 3738415) is deposited in the Missouri Botanical Garden Herbarium.

EXTRACTION AND FRACTIONATION.—Mature seeds were ground and extracted with MeOH in a Soxhlet apparatus, and the alkaloidal fraction was purified by ion-exchange chromatography on Dowex 50W-X8 (NH_4^+ form). Analysis of the per-TMSi derivative of the eluate by gc-ms showed the presence of five components with Rt's of 11.36 (8%), 11.70 (43%), 12.17 (6%), 12.47 (3%), and 14.29 (36%) min. The relative compositon was determined as area percent of the total ion chromatogram. The crude alkaloid fraction was further partitioned on a Dowex 50W-X8 (NH_4^+) ion-exchange column (1.5×60 cm) with gradient elution from 0 to 0.5 M NH_4OH (300 ml). Fractions (5 ml) were tested for inhibition of α-glucosidase and α- and β-mannosidase, exhibiting three bands of activity (API, APII, and APIII). APIII (fractions 40–55) was shown by gc-ms to consist primarily of 2-hydroxymethyl-3,4-dihydroxypyrrolidine [**3**] and fagomine [**5**] and was not examined further. Band API (fractions 23–32) and APII (fractions 33–39) were purified to homogeneity, as determined by analytical tlc and gc-ms, by preparative centrifugal tlc (16).

ISOLATION AND CHARACTERIZATION OF DEOXYMANNOJIRIMYCIN [**2**].—After further tlc purification, band API gave a peak with Rt 14.29 min on gc-ms of its TMSi derivative. Co-injection with an authentic sample of deoxymannojirimycin gave a single peak with the same retention time. Deoxynojirimycin gave a peak with Rt 14.91 min. Penta-TMSi-deoxymannojirimycin: eims *m/z* $[M]^+$ 523 (1), 508 (2), $[M-CH_2OTMSi]^+$ 420 (100), 330 (39), 258 (2), 240 (5). The ^1H- and ^{13}C-nmr spectra and optical rotation of the natural product were completely in accord with data previously reported for synthetic deoxymannojirimycin (31).

ISOLATION AND CHARACTERIZATION OF 6-DEOXY-DMDP [**1**].—Preparative centrifugal tlc purification of band APII gave a pale yellow oil, which gave a single peak with Rt 12.17 min on gc-ms of its TMSi derivative: eims *m/z* $[M]^+$ 435 (1), 420 (6), 332 (100), 242 (5), 133 (3), The oil solidified very slowly as a waxy material but could not be induced to crystallize from solution. $[\alpha]^{23}\lambda$ (c=1.10, MeOH) +26.2° (589), +27.2° (578), +30.6° (546); ^1H nmr (200 MHz, D_2O) δ 3.83 (1H, dd, $J_{3,4}=J_{4,5}=7.0$ Hz, H-4), 3.68 (1H, dd, $J_{5,7}=5.5$ Hz, $J_{gem}=-11.0$ Hz, H-7), 3.63 (1H, dd, $J_{5,7'}=7.0$ Hz, $J_{gem}=-11.0$ Hz, H-7′), 3.62 (1H, dd, $J_{2,3}=8.3$ Hz, $J_{3,4}=7.0$ Hz, H-3), 3.19 (1H, dt, $J_{4,5}=7.0$ Hz, $J_{5,7}=5.5$ Hz, $J_{5,7'}=7.0$ Hz, H-5), 2.97 (1H, dq, $J_{2,3}=8.3$ Hz, $J_{2,6}=6.7$ Hz, H-2), 1.20 (3H, d, $J_{2,6}=6.7$ Hz, -Me); ^{13}C nmr (50.3 MHz, D_2O) δ 86.0 (C-3), 80.8 (C-4), 65.1 (-CH_2OH), 64.3 (C-5), 58.5 (C-2), 19.9 (-CH_3); eims *m/z* $[M]^+$ 147 (4), 129 (2), $[M-CH_2OH]^+$ 116 (100), 99 (9) 98 (8), 87 (23), 69 (48), 60 (18), 44 (81); hrms *m/z* $[M]^+$ 147.0892 (calcd for $C_6H_{13}NO_3$, 147.0895). Synthetic 6-deoxy-DMDP (see Scheme 1): $[\alpha]^{23}\lambda$ (c=0.72, MeOH) +42.9° (589), +44.7° (578), +50.4° (546), +82.4° (436); ^1H and ^{13}C nmr identical to those of the natural compound. Gc-ms analysis of the TMSi derivative gave a peak at 12.17 min with ms identical to that of the natural compound. Co-injection of TMSi derivatives of the natural and synthetic compounds gave a single peak.

GLUCOSIDASE INHIBITION.—Amyloglucosidase (from *Aspergillus niger*), α-glucosidase (from yeast), β-

glucosidase (from almonds), α-galactosidase (from *As. niger*), β-galactosidase (from bovine liver), α-mannosidase (from jack bean), β-xylosidase (from *As. niger*), α-L-fucosidase (from bovine kidney), and all *p*-nitrophenyl glycoside substrates were purchased from either Boehringer Mannheim Biochemicals or the Sigma Chemical Company. β-Mannosidase was purified from *As. niger* as previously described (32). The enzymatic activities of the arylglycosidases were determined colorimetrically by monitoring the release of *p*-nitrophenol from the appropriate *p*-nitrophenyl glycoside substrate (33).

ACKNOWLEDGMENTS

We gratefully acknowledge the contribution of Mr. J. Michael Fay in providing the plant material. The assistance of Ms. Mabry Benson and Dr. William Haddon of the Western Regional Research Center in obtaining nuclear magnetic resonance spectra and mass spectrometric measurements, respectively, is appreciated. This research was supported in part by grants from the National Institutes of Health (HL-17783).

LITERATURE CITED

1. A.D. Elbein and R.J. Molyneux, in: "Alkaloids: Chemical and Biological Perspectives." Ed. by S.W. Pelletier, Wiley, New York, 1987, Vol. 5, pp. 1–54.
2. G.W.J. Fleet, L.E. Fellows, and B. Winchester, in: "Bioactive Compounds from Plants" (Ciba Foundation Symposium 154). Ed. by D.J. Chadwick and J. Marsh, Wiley, Chichester, 1990, pp. 112–122.
3. R.M. Polhill, in: "Advances in Legume Systematics." Ed. by R.M. Polhill and P.H. Raven, Royal Botanic Gardens, Kew, 1981, pp. 213–230.
4. L.D. Hohenschutz, E.A. Bell, P.J. Jewess, D.P. Leworthy, R.J. Pryce, E. Arnold, and J. Clardy, *Phytochemistry,* **20**, 811 (1981).
5. R. Saul, J.P. Chambers, R.J. Molyneux, and A.D. Elbein, *Arch. Biochem. Biophys.*, **221**, 593 (1983).
6. R. Saul, R.J. Molyneux, and A.D. Elbein, *Arch. Biochem. Biophys.*, **230**, 668 (1984).
7. B.D. Walker, M. Kowalski, W.C. Rosen, L.R. Rohrschneider, W.A. Haseltine, and J. Sodroski, *Proc. Nat. Acad. Sci. USA,* **84**, 8120 (1987).
8. R.A. Gruters, J.J. Neefjes, M. Tersmette, R.E.Y. de Goede, A. Tulp, H.G. Huisman, F. Miedema, and H.L. Ploegh, *Nature,* **330**, 74 (1987).
9. A.S. Tyms, E.M. Berrie, T.A. Ryder, R.J. Nash, M.P. Hegarty, M.A. Mobbereley, J.M. Davis, E.A. Bell, D.J. Jeffries, D. Taylor-Robinson, and L.E. Fellows, *Lancet,* 1025 (1987).
10. P.S. Sunkara, T.L. Bowlin, P.S. Liu, and A. Sjoerdsma, *Biochem. Biophys. Res. Commun.*, **148**, 206 (1987).
11. B.L. Rhinehart, K.M. Robinson, A.J. Payne, M.E. Wheatley, J.L. Fisher, P.S. Liu, and W. Cheng, *Life Sci.*, **41**, 2325 (1987).
12. D.L. Dreyer, K.C. Jones, and R.J. Molyneux, *J. Chem. Ecol.*, **11**, 1045 (1985).
13. R.J. Molyneux, J.N. Roitman, G. Dunnheim, T. Szumilo, and A.D. Elbein, *Arch. Biochem. Biophys.*, **251**, 450 (1987).
14. R.J. Molyneux, Y.T. Pan, J.E. Tropea, M. Benson, G.P. Kaushal, and A.D. Elbein, *Biochemistry,* **30**, 9981 (1991).
15. R.J. Molyneux, J.E. Tropea, and A.D. Elbein, *J. Nat. Prod.*, **53**, 609 (1990).
16. R.J. Molyneux, M. Benson, R.Y. Wong, J.E. Tropea, and A.D. Elbein, *J. Nat. Prod.*, **51**, 1198 (1988).
17. R.J. Nash, L.E. Fellows, A.C. Plant, G.W.J. Fleet, A.E. Derome, P.D. Baird, M.P. Hegarty, and A.M. Scofield, *Tetrahedron,* **44**, 5959 (1988).
18. C.M. Harris, T.M. Harris, R.J. Molyneux, J.E. Tropea, and A.D. Elbein, *Tetrahedron Lett.*, **30**, 5685 (1989).
19. S.V. Evans, A.R. Hayman, L.E. Fellows, T.K.M. Shing, A.E. Derome, and G.W.J. Fleet, *Tetrahedron Lett.*, **26**, 1465 (1985).
20. R.J. Nash, E.A. Bell, and J.M. Williams, *Phytochemistry,* **24**, 1620 (1985).
21. R.J. Molyneux, L.F. James, K.E. Panter, and M.H. Ralphs, *Phytochem. Anal.*, **2**, 125 (1991).
22. J. Furukawa, S. Okuda, K. Saito, and S.-I. Hatanaka, *Phytochemistry,* **24**, 593 (1985).
23. G.W.J. Fleet, S.J. Nicholas, P.W. Smith, S.V. Evans, L.E. Fellows, and R.J. Nash, *Tetrahedron Lett.*, **26**, 3127 (1985).
24. D.W.C. Jones, R.J. Nash, E.A. Bell, and J.M. Williams, *Tetrahedron Lett.*, **26**, 3125 (1985).
25. L.E. Fellows, E.A. Bell, D.G. Lynn, F. Pilkiewicz, I. Miura, and K. Nakanishi, *J. Chem. Soc., Chem. Commun.*, 977 (1979).
26. M. Koyama and S. Sakamura, *Agric. Biol. Chem.*, **38**, 1111 (1974).
27. A. Welter, J. Jadot, G. Dardenne, M. Marlier, and J. Casimir, *Phytochemistry,* **15**, 747 (1976).
28. M.L. Wolfrom and S. Hanessian, *J. Org. Chem.*, **27**, 1800 (1962).

29. S. Czernecki, C. Georgoulis, and C. Provelenghiou, *Tetrahedron Lett.*, **50**, 4841 (1979).
30. B. Winchester, C. Barker, S. Baines, G.S. Jacob, S.K. Namgoong, and G.W.J. Fleet, *Biochem. J.*, **265**, 277 (1990).
31. G.W.J. Fleet, L.E. Fellows, and P.W. Smith, *Tetrahedron,* **43**, 979 (1987).
32. A.D. Elbein, S. Ayda, and Y.C. Lee, *J. Biol. Chem.*, **252**, 2206 (1977).
33. J.E. Tropea, R.J. Molyneux, G.P. Kaushal, Y.T. Pan, M. Mitchell, and A.D. Elbein, *Biochemistry,* **28**, 2027 (1989).

Received 22 January 1993