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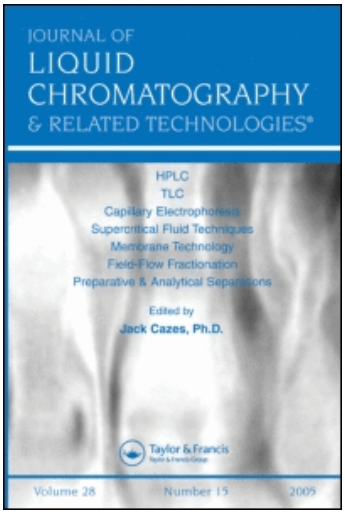

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An Improved Method for The Isolation of the Lignan Constituents of *Saururus Cernuus* by Reverse Phase Column Chromatography

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AN IMPROVED METHOD FOR THE ISOLATION OF THE LIGNAN CONSTITUENTS OF *SAURURUS CERNUUS* BY REVERSE PHASE COLUMN CHROMATOGRAPHY

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ABSTRACT

The above-ground parts of the aquatic weed, *Saururus cernuus* contain many constituents with varying and significant biological acitivities. In the earlier method of isolation, the concentrated methanolic extract was fractionated by a 2-3 step solvent partition, and the appropriate fractions subjected to two or three steps of chromatography, as well as other manipulations, which together, made the isolation a somewhat elaborate and time-consuming operation, especially when a medium to large-scale extraction is to be carried out.

In an effort to simplify this process, the methanolic extract concentrate is now partitioned between benzene and water, and the organic extract subjected directly to reverse phase column chromatography. Most of the lignan constituents could be isolated either directly, or after one small additional column, where necessary.

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This method has some distinct advantages over the earlier scheme. Besides being simpler, and more readily applicable for large-scale work-up, the chlorophylls and other liipophilic components, which usually contaminate the lignan fractions in the normal phase silica column, remain on the column until almost all of the lignan constituents are eluted, thus aiding the purification.

The previously obtained lignan components, can all be isolated readily by the use of this simplified procedure.

INTRODUCTION

The aquatic weed, *Saururus cernuus* L (N.O. Saururaceae) which grows mainly in the eastern United States, was known and used during the 19th century for its anti-inflammatory activity.[1,2] A systematic study was undertaken in our laboratory which yielded a number of novel lignan and other constituents.[3-6] The most important of these were the dineolignan type compounds named manassantins A and B (**1** and **2**), which showed potent neuroleptic acitivity.[7-9]

Because of the continued interest in this and other activities found in this plant, a reexamination of the isolation process used earlier was undertaken, with the objective of simplifying and streamlining the process, and for possible large scale applicability. Based on earlier, successful applications of a reverse phase column technique for the fractionation of the crude extracts of *Taxus brevifolia*, for the isolation of paclitaxel and several of its analogues,[10,11] use of this principle was studied with the extracts of *Saururus cernuus*, and the details of these studies are provided in this communication.

MATERIALS AND METHODS

Plant Material

The above-ground parts of the plant (which has been previously identified by the University of Florida Herbarium, where a voucher specimen was submitted) are collected locally, in and around Gainesville, FL during May-September, when the plant is found most commonly, dried in the sun and stored until needed for extraction.

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Extraction and Partition

The dried plant material was ground to a coarse mesh (0.5-1 cm) and extracted in 25 lb quantities in a stainless steel tank using methanol. The extract was drained after 24 h and the extraction was repeated 3-4 more times, using absorbance at 275 nm as a guide, to indicate the concentration of extractables. Usually four extractions gave most of the desired constituents. The extracts were concentrated under reduced pressure to a thick green syrup, which was partitioned between water (5 gal) and benzene (5 gal). The organic layer was separated and the aqueous layer extracted a second time using benzene (3 gal). The aqueous layer was concentrated to a syrup and set aside for further study. The combined benzene extract which contained the bulk of the absorbance at 275 nm, representing the lignan constituents was concentrated to a dark green semi-solid (5% of the weight of the dried plant), referred to as the "extract solids" and stored until use.

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Chromatography

A column was set up using C_{18} bonded silica gel (approximately 800g, 15-35 micron size, Phase Separations, Inc., Norwalk, CT) using methanol, in a threaded glass column of the Mitchell-Miller type (2.5 x 24", Ace Glass Co. Vineland, NJ), suitable for low pressure liquid chromatography). The column was equilibrated with 40% methanol in water, which made it ready for use.

The extract solids (150 g) was dissolved in methanol (450mL) by warming if necessary, and to this solution was added approximately a 150g equivalent of the equilibrated silica gel (about 15-20% of the silica from the above column) with stirring. While the stirring is being continued, 400 mL of the 40% methanol in water was added, followed by water (600mL), to make an approximate total volume of 1500 mL. The mixture was warmed in a water bath to approximately 50°C and the stirring continued until there was no visible green precipitate or oily material, and an aliquot of the silica gel/sample slurry, taken in a test tube, settled readily to give a relatively clear supernatant. The slurry was then filtered using light suction and the solid, re-slurried using part of the filtrate, and the thick slurry added to the column.

The clear filtrate was then pumped on to the top of the column using a metering pump (Eldex-Fisher Scientific Co.). From time to time, the column feed was checked to see that it remained clear, and if not, to either warm briefly or add minimal amounts of methanol to it, until it became clear, so as to prevent blockage of the pump.

Following the addition of the sample, the column was eluted with a step gradient of methanol-water (50, 55, 60, 65, 75, and 85% methanol). Fractions (200mL) were collected and monitored by uv absorbance (275 nm) and TLC. The change to the next concentration of the solvent was determined by the results of the monitoring of the fractions. For example, when the absorbance values rose as a result of the previous change, the solvent was continued until a definite trend to lower values was seen. Similarly, when the TLC showed the trend towards decreasing intensity of the major spot, and no new spot has shown a tendency to increase, the solvent is changed to the next level. In general, 2-4 multiples of the hold-up volumes of the column were used. After the 85% methanol-water was completed, the solvent was changed to 100% methanol, which was later changed to a mixture of methanol, ethyl acetate and ligroin (2:1:1). Most of the chlorophylls and other lipid-soluble components which were held up on the column during the run, started eluting when the 100% methanol was started, and the three-solvent mixture hastened their elution. After the column has been thus completely stripped of the green color, it was washed with methanol, equilibrated with 40% methanol in water, and is made ready for another chromatographic run.

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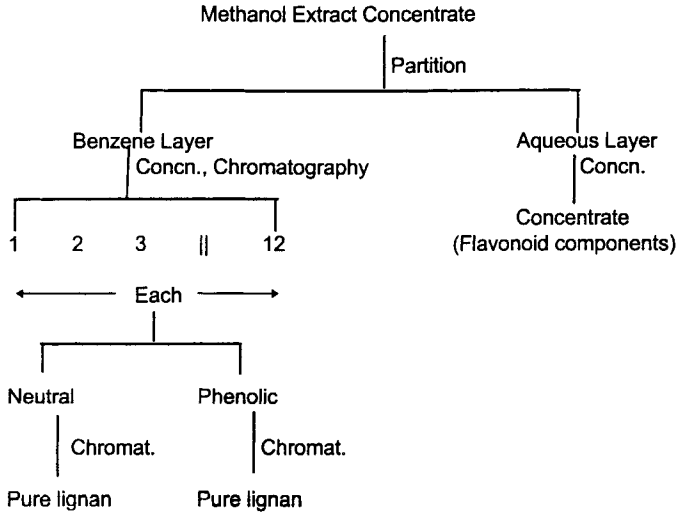
Based on the monitoring data, the fractions were combined into small groups (3-5 fractions) and concentrated to an oil and set aside for further work. The concentrates were examined by TLC to study the number, relative proportions, and nature of the components, eg. neutral, phenolic, lignan, non-lignan, etc. Some of the major lignan constituents isolated earlier[3-6] from this plant, such as austrobailignan-5 (most lipophilic), saucernetin, saucerneol (phenolic), manassantin B, and manassantin A (most hydrophilic), were used as markers to orient the others on the TLC.

The appropriate concentrates from above, were each partitioned counter-currently, using two separatory funnels, and the solvent system: methanol, 0.2 N aqueous sodium hydroxide, benzene and ligroin (1:1:1:1), to separate the phenolic and the neutral components. The aqueous methanolic layers containing the phenolic fractions were partially concentrated, neutralized, and extracted with benzene. The neutral and phenolic fractions thus separated, were each subjected to a brief column on normal phase silica for final separation and purification.

EXPERIMENTAL

Analytical HPLC was performed using two different units. For routine use, a combination of Waters 501 pump, with a U6K injector, a 486 tunable absorbance detector, and a Goertz Servogor 120 recorder was used. For determination of purity and quantitative information on composition etc., a setup containing a Waters 600 E

Scheme for the Fractionation of the *Saururus* Extract



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pump with gradient control system, a 996 photodiode array detector, a 717 autosampler coupled with an NEC-386 computer and printer was used. Waters Millenium 2.1 program was used with the instrument. Standard columns (4.6 mm x 25 cm, Whatman, Partisil) packed with C_8-bonded silica (5 micron diameter) were used with either of the solvents: 60% acetonitrile-water or 60% acetonitrile, 10% methanol and water. The flow rate for both columns was 0.5 mL per min. The detector was set at 275 nm.

Thin-layer chromatography was carried out using silica gel HF-60, 254+366 (EM Science/Fisher) coated on microscope slides, by pouring as a slurry and drying the slides in an oven. The solvent systems consisted of 10-20% acetone/benzene, 5-10% methanol/dichloromethane, or 20-40% ethyl acetate/ligroin. Visualization was by a uv lamp, and by charring with 1 N H_2SO_4 in which most of the lignan constituents gave a scarlet red color, whereas those lignans containing methylenedioxy groups gave a violet brown color. Quantitative TLC was run using 2x3" slides on which the sample was applied as a band and developed. The bands were each scraped out, eluted with methanol, and the absorbance and the uv spectrum run on each, directly and after addition of a base to determine shifts due to phenolic compoumds. Column chromatography was carried out using silica gel (Fisher 100-200 mesh or 235-425 mesh, with a solvent sequence of ligroin $\rightarrow$ benzene $\rightarrow$ benzene with acetone (5-10%) and benzene with methanol (5-10%).

Alternatively, mixtures of ligroin and ethyl acetate were also used. Melting points were determined on Fisher-Johns apparatus and were uncorrected. The following instrumentation was used to record the spectra described here: uv, Perkin-Elmer Lambda 3B; ir, Perkin-Elmer PE-1420; and nmr, Varian VXR-300, Varian Gemini-300 and General Electric QE-300 spectrometers. The nmr spectra were recorded in δ values using TMS as an internal standard. The assignments were made with the help of COSY, HETCOR and APT spectra. Mass spectra (FAB) were obtained on a Finnegan Mat 95Q spectrometer using a cesium gun operated at 15 Kev of energy.

RESULTS

Before discussing the elution sequence for the components from the reverse phase column, it may be noted that a total of nearly 11 lignan components were obtained pure from this column to illustrate the applicability of the procedure. There are several other minor components which can also be isolated. An HPLC trace of these standards along with that of the extract solids is shown in Fig.1. The elution profile as seen by the absorbance values at 275 nm is shown in Fig. 2.

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The elution of the lignans started, as the 50% methanol/water was started, and continued until the 85% methanol/water was completed. The elution sequence and the respective yields of the various lignans are shown in Table 1. The major peak in the elution profile based on the uv absorbance (Fig. 2) was found to be due to a non-lignan compound which is not investigated here further. Of the various lignans that were obtained and characterized (the bold numbers refer to the structures which are shown in Fig. 3), austrobailignan-5 **11** appears to be the major lignan component of the extract.

Characterization of the Major Lignans from *Saururus*

Bis-demethyl saucernetin **3**: Fractions 11-20 were combined, concentrated to an oil (1.5 g) and subjected to partition in the solvent system described under Materials and Methods. The phenolic fraction (0.6 g) was purified by a small silica column (25 g) in benzene/ligroin (1:1). Elution with 2% acetone in benzene gave the major component, obtained as a colorless powder, yield, 0.15 g, ^{1}H nmr, (δ): 0.68, d, J=6.6 Hz, 6H, H-9,9'; 2.24, m, 2H, H-8,8'; 3.87, s, 6H, 2xOMe; 5.42, d, J=6 Hz,2H, H-7,7'; 6.75-6.90, m, 6H, H-Ar.

The above compound was characterized by methylation carried out in acetone by refluxing with dimethyl sulfate and potassium carbonate to yield the dimethyl ether which was crystallized and found to be identical with saucernetin[3].

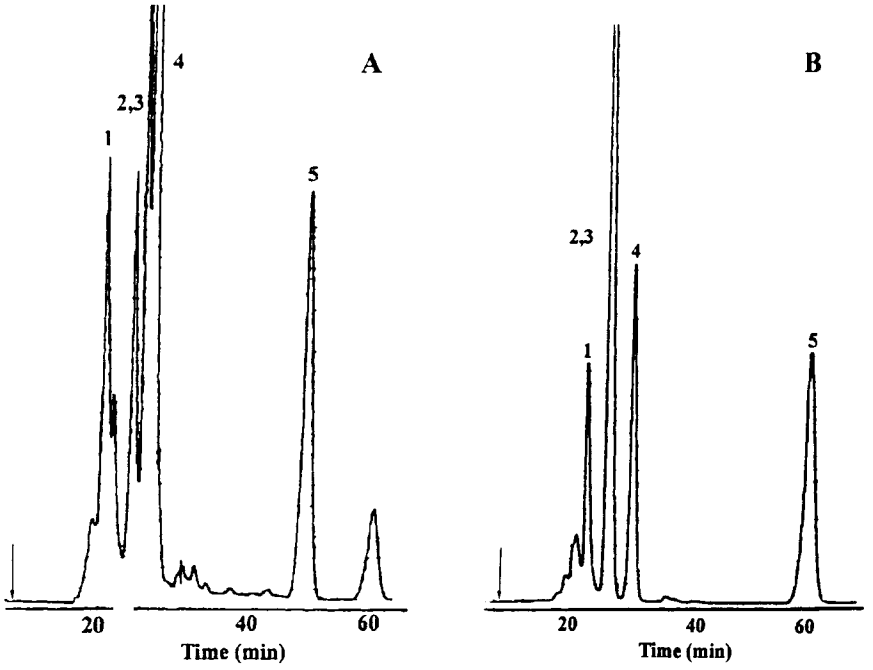

Figure 1. HPLC Trace of A) Extract of Saururus, B) Mixture of Sc-1, SC-2, SC-6, SC-7 and SC-8. 1: Manassantin A (**1**); 2: Saucerneol (**7**); 3: Manassantin B (**2**);4: Saucernetin (**5**), 5: Austrobailignan-5 (**11**).

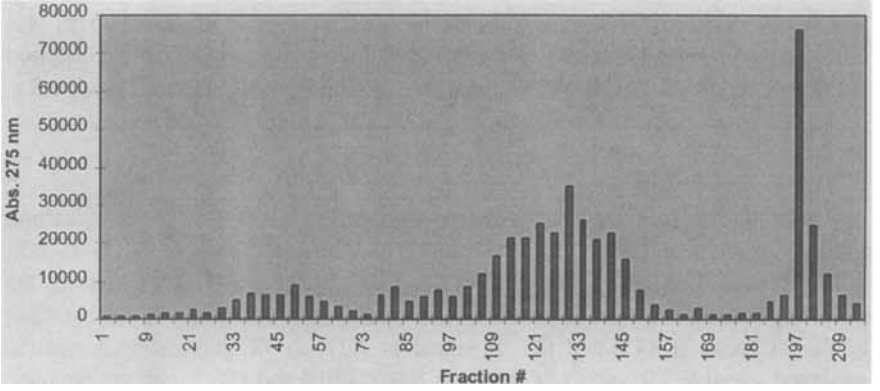

Figure 2. Elution Profile of the Extract of *Saururus*.

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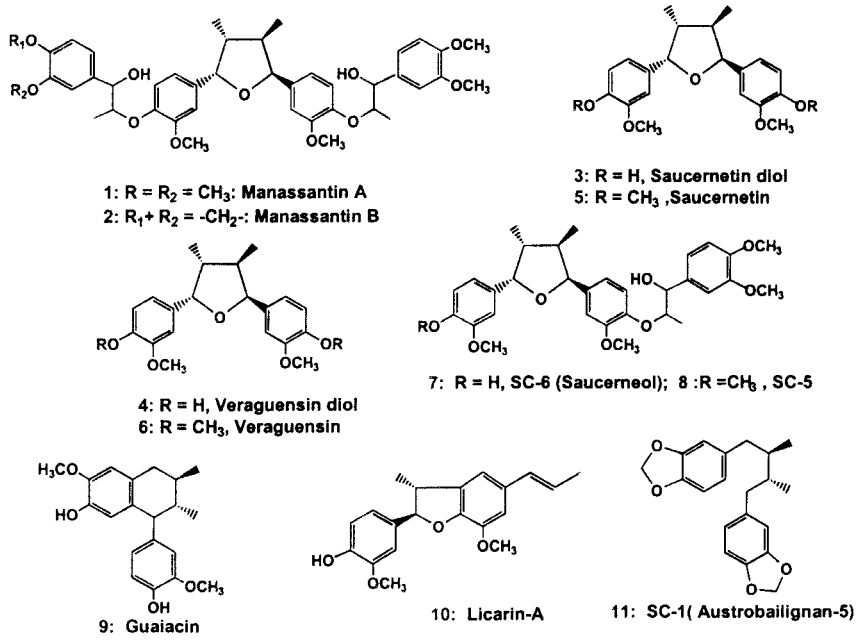

Figure 3. Structures of some *Saururus* lignans.

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Bis-demethyl veraguensin **4**: Fractions 21-30 processed by the method given under 3. The major fraction from the column yielded **4** as a colorless glassy solid, yield, 0.1 g. Its nmr spectral characteristics were identical with those reported.[12,13] Saucernetin **5**: Fractions 36-50 were combined and concentrated to an oil (6 g) which was subjected to the phenolic partition. The neutral fraction (2.5 g) was chromatographed on a normal phase silica column in benzene/ligroin (1:1). Elution with benzene gave **5**, which was obtained as a colorless crystalline solid, yield, 1.5 g. Its physical and spectral properties were identical with those of saucernetin described earlier.

The proton nmr spectrum was reported earlier[3], but not the ^{13}C spectrum, which is given here: (δ) 14.73, C-9,9'; 44.02, C-8,8'; 55.88, 2xOMe, 83.51, C-7,7'; 109.6, 110.8, 118.4, 134.0, 147.9, 148.6, C-Ar. In the HETCOR spectrum, the following interactions were observed: a) the ^{13}C signal at 14.7 and ^{1}H peak at 0.69, b) the ^{13}C peak at 44.0 and the ^{1}H signal at 2.27, c) ^{13}C peak at 55.9 and the methoxyl signal at 3.9, and d) ^{13}C peaks at 109.6, 110.8 and 118.4 with the aromatic proton signals at 6.9.

Table 1

Elution Sequence of the Saururus Lignans

	Compound	Yield
1	SC-2 diol **3**	0.15 g
2	SC-3 diol **4**	0.1 g
3	SC-2 **5**	1.5 g
4	SC-3 **6**	0.1g
5	SC-6 **7**	0.9 g
6	SC-5 **8**	0.3 g
7	SC-8 **1**	3.1 g
8	Guaiacin **9**	0.25 g
9	Licarin **10**	0.2 g
10	SC-7 **2**	0.3 g
11	SC-1 **11**	15 g

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Veraguensin **6**: Elution of the above column with 2% acetone in benzene gave the minor component **6**, as a colorless crystalline solid whose physical and spectral properties were identical with those reported earlier for veraguensin[3]. Saucerneol **7**: The phenolic fraction (3 g) obtained from fractions 36-50 as given under **5** was purified by chromatography on a normal phase silica column using benzene. Elution with 5% acetone/benzene gave **7**, obtained as a colorless powder, yield, 1.6 g. Its physical and ^{1}H nmr spectral properties were identical with those reported earlier.[4] The ^{13}C nmr spectrum has not been reported before and is given here: δ 14.8, C-9,9'; 16.9, C-9''; 44.1, C-8,8'; 55.9, OMe; 78.4, C-7''; 83.5, C-7,7'; 84.0, C-7''; 108.9, 110.1, 110.9, 113.9, 118.7, 119.0, 119.9, 132.5, 133.8, 136.6, 144.5, 146.2, 146.4, 149.0, 150.5, C-Ar.

Saucerneol methyl ether (SC-5, **8**): Fractions 66-75 were combined and concentrated (2 g). It was freed from the phenolic components by base partition. The neutral fraction (1 g) was purified by chromatography on silica gel in benzene.

The major component **8** was obtained by elution with 2% acetone in benzene, as a colorless powder, yield, 0.4 g), ^{1}H nmr spectrum: 0.70, d, J=6 Hz, 3H, H-9; 0.72, d, J=6 Hz, 3H, H-9'; 1.17, d, J=6Hz, 3H, H-9''; 2.25, d, 2H, H-8,8'; 3.90, 3.93, s, 15H, 5xOMe; 4.13, m, 1H, H-8''; 4.65, d, J=8.1 Hz, 1H, H-7''; 5.46, d, J=6 Hz, 2H, H-7,7'; 6.8-7.0, m, 9H, H-Ar; ^{13}C nmr spectrum, 14.8, C-9,9'; 16.9, C-9''; 44.0, C-8,8'; 55.9, OMe; 78.3, C-7''; 83.3, C-7,7'; 109.6, 110.0, 110.1, 110.8, 118.7, 119.9, 132.2, 132.6, 136.6, 146.6, 147.9, 148.6, 148.98, 148.9, 150.5, C-Ar.

The spectral data indicated that the compound **8** was the methyl ether of saucerneol. This was confirmed by methylating saucerneol in acetone with dimethyl sulfate and potassium carbonate and showing that the product was identical with **8**.

Manassantin A **1**: Fractions 76-95 were combined, concentrated (12 g), and partitioned to free it from any phenolic components. The neutral fraction (8 g) was chromatographed on silica gel (175 g) using benzene. Elution with 5% acetone in benzene gave 1 as a colorless powder, yield, 4.5 g. Its ^{1}H and ^{13}C nmr spectral properties were identical with those described.[4]

Licarin **10**: The phenolic components (2.7 g) from fractions 76-95 were purified by chromatography on silica using benzene. Elution with benzene gave **10** as a colorless crystalline solid. Its physical and spectral properties were identical with those described earlier.[14]

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Guaiacin **9**: From the column described under **10**, elution with 2% acetone in benzene gave **9**, obtained as a colorless crystalline solid, the physical and spectral properties of which were identical with those described earlier.[15]

Manassantin B **2**: Fractions 96-104 were combined, concentrated and freed from phenolic components. The neutral fraction (1.5 g) was purified by chromatography on silica gel using benzene. Elution with 5% acetone in benzene gave **2** as a colorless powder, yield, 0.6 g; ^{1}H nmr spectrum, 0.72, d, 6 Hz, 6H, H-9,9'; 1.15, 1.17, d, J=6 Hz, H-9'', 9'''; 2.29, m, 2H, H-8,8'; 3.87, 3.89, 3.92, 3.93, s, 4xOMe; 4.11, m, 2H, 8'', 8'''; 4.62, 4.64, d, J=9 Hz, 2H, 7'',7'''; 5.46, d, J=6 Hz, 2H, H-7,7'; 5.94, s, 2H, O-CH_2-O; 6.78-7.00, m, 12 H, H-Ar; ^{13}C nmr, 14.8, 14.8, C-9, 9'; 16.8, 17.0, C-9'',9'''; 44.1, 44.1, C-8,8'; 55.8, O-Me; 78.3, 78.3, C-7'', 7'''; 83.3, 83.3, C-7, 7'; 83.8, 83.9, C-8'', 8'''; 100.9, OCH_2O; 108.0, 107.5, C-5, 5'; 110.1, 110.1, 110.1, 110.9, C-2, 2' 2'', 2'''; 118.6, 118.6, C-6,6'; 119.9, 120.9, C-6'', 6'''; 118.6, 118.8, C-5'', C-5'''; 136.4, 136.5, 132.6, 134.0, C-1,1', 1'', 1'''; 146.2, 146.4, 147.3, 147.7, C-3, 3', 3'', 3'''; 150.2, 150.5, 148.8, 149.0, C-4, 4', 4'', 4'''.

Austrobailignan 5 **11**: Fractions 191-205 were combined, concentrated (30 g) and subjected to chromatography on silica using 1:1 benzene/ligroin. The major component was obtained by elution with 3:1 benzene/ligroin, and it was obtained as a colorless crystalline solid, whose physical and ^{1}H spectral properties agreed with those described earlier.[16,3] Its ^{13}C nmr spectrum was not described in the past and it is given here: (δ), 13.8, C-9,9'; 38.2, C-8,8'; 41.1, C-7,7'; 100.7, O-CH_2-O; 107.9, C-5,5'; 109.8, C-2,2'; 121.7, C-6,6'; 133.4, C-1,1'; 145.4 C-4,4'; 147.4 C-3,3'.

DISCUSSION

The order of elution of the compounds from the reverse phase column (Table 1) deserves some comment. The order does not seem to correlate entirely with either the polarity, molecular weight, or the mobility on TLC on normal phase silica. For example, SC-1 (**11**) (austrobailignan-5) has the highest R_f (1.0, most lipophilic) of the lignans of *Saururus* as seen in TLC (10-20% acetone in benzene), followed by those of intermediate R_f (0.6-0.7): SC2 (**5**) (saucernetin), SC-3 (**6**) (veraguensin) and guaiacin (**9**). Significantly slower than these are the sesqui and dilignans such as SC-5 (**8**), SC-6 (**7**), SC-7 (**2**) and SC-8 (**1**), which appear in that order in the TLC (R_f 0.1-0.2). In the reverse phase column, as expected, SC-1 (**11**) was the last to be eluted. However, SC-2 (**5**) and 3 (**6**) (with higher R_f values than SC-6 (**7**), -7(**2**) and -8 (**1**) emerge from the column before SC-8, 6 and 7. Also, compounds that show the same R_f in TLC are separated significantly in the reverse phase column, which is seen often.

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The procedure described here for the processing of the extract of *Saururus* using the reverse phase column, clearly has some advantages over the one based on the use of a normal phase silica columns. Among the advantages, the following are important to note. First, it involves fewer steps than were used before, because the two to three stage solvent partitions can be replaced by one partition step (water/benzene), which separates the water-soluble flavonoid glycosides from the lipophilic lignans and other constituents. By applying the concentrated benzene extract directly on the reverse phase column, the need for handling the lignan constituents in two or three subgroups is eliminated. In spite of the more complex mixture that is being applied to the C_{18} bonded silica column, the resolution was quite satisfactory. Secondly, in the normal phase silica column, the chlorphylls and carotenoid components were partially eluted as the eluting solvent changed each time, such that, most fractions when concentrated were greenish yellow, whereas, in the reverse phase column this leaching of the pigments was to a much smaller extent, and whatever amounts of these pigments present in these samples could be readily removed when they are subjected to the second (silica) column. Thus, the noncrystalline lignans such as SC-5, -6, -7 and -8 could be readily obtained as homogeneous, colorless solids with much less effort.

One of the difficulties in the normal phase silica column used earlier process was the co-elution of some of the sesqui and dineolignans (SC-5, -6, -7 and -8). Thus, even after the removal of SC-6 by the phenolic partition, the elution pattern of SC-5, 7 and 8 still gave mixtures such as SC-5 with decreasing amounts of SC-7, and SC-7 with decreasing amounts of SC-8. To obtain the last two in pure form, it was necessary to acetylate the mixture, separate the acetates, and regenerate the original compounds by saponification. In the current procedure, this was not

necessary. SC-8 and SC-7 separated well from each other, actually requiring a different solvent mixture for the elution of each. Most of the fractions showed single spots, while those which may contain mixtures can be purified readily in the subsequent "small" column. Similarly, many of the fractions obtained earlier by the normal phase silica column, which were assumed to be SC-7, were found by nmr spectra (only the latter having the methylenedioxy signal at δ 5.9) to be mixtures of SC-6 and SC-7. These mixtures could be readily separated by the reverse phase column, with SC-6 eluting first and SC-7, later, after a change of the solvent (see figs). The important overall result was that even though relatively large quantities (100's of pounds) of the plant were extracted before, only a portion of the earlier fractions could be taken up to homogeneity, the rest being left as mixtures. In this current procedure, these mixtures, as well as new extractions, could be processed readily, such that some of these components could be obtained for the first time, in 10-50 g quantities in a relatively short time.

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The fractions from the initial reverse phase column, are combined based on the TLC and HPLC data, separated into the neutral and phenolic components. This type of neutral/phenolic separation is best done at this stage rather than at the original total extract stage. The phenolic lignans do not partition into aqueous base from solvents such as dichloromethane or even benzene. In order to separate most of the phenolics from the neutral components, it is necessary to use a solvent system listed under Materials and Methods, in which the benzene is diluted with ligroin (1:1) to lower the solubility of the ionized phenols, and methanol is added to the aqueous layer to increase the solubility of the ionized phenols. The partition also works best when one is using dilute solutions and thus, to do this at the original crude extract stage will require rather large volumes, and it is not convenient. Even when carried out under optimal conditions, some phenolic lignans still partially remain in the organic layer, eg. licarin and SC-6.

After the phenolic partition, the samples are subjected to the second column. The column used is usually a much smaller column, and can be either a reverse phase, or a normal phase column. At this point, other reverse phase column packings such as the CN, phenyl, etc. bonded silica column can offer specific advantages for resolution and thus can be used. Such information may be obtained during the HPLC analyses using columns containing these packings. Similar choices may be made for the normal phase column between various types of silica, florisil etc.

One of the most important advantages of the new procedure is its adaptability to larger scale operations. Based on the earlier experience with the processing of the extracts of *Taxus brevifolia*, the laboratory size columns on 150-200 g of the crude

extract could be scaled up to 2.3-2.7 kg of the extract using a 6"x6' column. Judging from the ease of preparing the sample and applying to the column in the present case, similar scale-up can be carried out on the extracts of *Saururus* if such need arises. The solvents used such as methanol/water mixtures, as well as the fact that the column can be used again and again, offer an economic advantage. Lastly, unlike the case with a normal phase silica or florisil columns, on a reverse phase column, none of the components of the extract can be "lost" due to irreversible adsorption on the silica.

The most important part of the process is the preparation of the sample for the reverse phase column. In most applications of preparative reverse phase chromatography reported in the literature, where this step is either the last or next to the last step, the sample is already of high degree of purity. In the current process, the reverse phase column is the first step in the purification scheme, and hence the sample is the crude extract itself. Because of its highly lipid soluble nature, the sample has little or no solubility in the solvent to be used, 40% methanol in water. Thus, it has to be applied as a suspension, which may suggest many problems because of the nature of lipophilic impurities such as chlrophylls, carotenoids, waxes, and such, normally present in the plant extracts. It is, thus, difficult to believe that such a column can perform satisfactorily. However, the C-8, or the C_{18} bound silica seems to absorb (or even dissolve) this lipophilic material so that no free oily or waxy material is left after the sample preparation as described, and the slurry is easily applied. The column performs as though a soluble sample has been used.

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REFERENCES

1. D. L. Phares, Am. J. Pharm., **39**, 468, (1867).

2. F. P. Porchier, **Resources of the Southern Fields and Forests, Medical, Economical and Agricultural**; Walker Evans, and Cogswell, Charleston, (1869).

3. K. V. Rao, F. M. Alvarez, J. Nat. Prod., **45**, 393-397 (1982).

4. K. V. Rao, F. M. Alvarez, Tetrahedron Lett., 4947-4950 (1984).

5. K. V. Rao, N. S. Prakasa Rao, J. Nat. Prod., **53**, 212-215 (1990).

6. K. V. Rao, G. S. Reddy, J. Nat. Prod., **53**, 309-312 (1990).

7. K. V. Rao, V. N. Puri, P. K. Diwan, F. M. Alvarez, Pharmacol. Res. Commun., 19, 629-638 (1987).

8. K. V. Rao, V. N. Puri, Life Sci., **42**, 2717-2720 (1988).

9. K. V. Rao, V. N. Puri, H. S. El-Sawaf, Europ. J. Pharmacol., **179**, 367-376 (1990).

10. K. V. Rao, J. B. Hanuman, C. Alvarez, M. Stoy, J. Juchum, R. M. Davies, R. Baxley, Pharm. Res., **12**, 1003-1010 (1995).

11. K.V. Rao, R. S. Bhakuni, J. Juchum, R. M. Davies, J. Liq. Chrom. & Rel. Technol., **19**, 427-447 (1996).

12. G. Schmedia-Hirschmann, F. Tsichritzis, J. Jakupovic, Phytochemistry, **31**, 1731-1735 (1992).

13. P. K. Agrawal, A. K. Pathak, Magnetic Resonance in Chemistry, **32** 753-773, (1994).

14. C. J. Aiba, R. G. C. Correa, O. R. Gottleib, Phytochemistry, **12**, 1163-1166 (1973).

15. P. L. Majumdar, A. Chatterjee, G. C. Sengupta, Phytochemistry, **11**, 811-813 (1972).

16. S. T. Murphy, E. Ritchie, W. C. Taylor, Australian J. Chemistry, **28**, 81-84 (1975).

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