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Countercurrent Chromatography Fractions of Plant Extracts with Anti-Tuberculosis Activity

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Countercurrent Chromatography Fractions of Plant Extracts with Anti-Tuberculosis Activity

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Abstract and Acknowledgements

Samples of numerous plant species were received from the southwestern part of the USA, from Richard Spjut, and plant samples were collected here in Illinois. All were extracted with typical solvents, giving crude residues, some of which were subjected to chromatographic methods. Some of the crude residues and some of the fractions were tested for anti-tuberculosis activity and/or antibacterial activity. Test results are given below.

Introduction

In a general way, bioactive natural products are dealt with very well by Liang & Fang, 2006 (See “References” section below.) More specifically, the southwestern part of the United States has a large variety of indigenous plants many of which have not been investigated for their medicinal potential, and only very few have had their extracts separated into the individual compounds they may contain. But, some information is available for Native American herbal uses (Moerman, 2003).

Methods

Dry samples of plant materials were ground into powder and were extracted in a Soxhlex apparatus with hexane or dichloromethane. The extracted solid samples were further extracted using methanol. The solutions were then concentrated using a rotavapor, under reduced pressure at about 50-60 ° C, until no more solvent would come off, giving crude residues, some of which were submitted for biological testing without purification. Of course the strategy was to try to figure out which ones (if any) might be worthy of separation into individual chromatographic fractions, which could also be tested. Countercurrent chromatography (CCC) was run on two sw/usa crude residues (DA-1 and DA-17) by Dr. Brent Friesen. One crude Illinois residue was subjected to open flash column chromatography (FCC) on silica gel, with the assistance and direction of Jordan Gunn. We sent crude residues and selected fractions to the Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, where they were tested for anti-tuberculosis activity, by Baojie Wan under the direction of Dr. Scott G. Franzblau, as shown in the “Anti-TB Data” table. Also, we sent crude sw/usa residues to the University of Notre Dame for antibacterial testing, under the direction of Dr. Shahriar Mobashery, as shown in the “Antibacterial Data” table.

Anti-TB Data

Sample	MABA MIC [ug/ml]	LORA MIC [ug/ml]
DA-1*	24.6	41.9
DA-19 (fraction 65 of CCC of DA-1)	47.0	
DA-17*	>50	
DA-3 (fraction 19 of CCC of DA-17)	48.1	
DA-12*	22.7	
P8	43.1	

*Crude sw/usa residues

Standards	MIC [uM]	MIC [uM]
RMP	0.04	0.76
INH	0.45	> 128
PA824	0.91	1.57

MABA (Microplate Alamar Blue Assay)
LORA (Low Oxygen Recovery Assay)

Antibacterial Data

MICs in µg/mL			
Sample	S. aureus	P. aeruginosa	E. faecium
31-1	256	>256	64
60-1	64	256	64
62-4	32	128	16
64-3	32	128	16

Results, Conclusions, and Further Work

We applied CCC only to sw/usa samples. We submitted one of our active crude sw/usa residues, DA-1, to CCC, and had selected fractions tested for anti-TB activity, and we compared with known anti-TB compounds (RMP, INH, and PA824). Since at least one of those fractions (fr. #65), DA-19, had some MABA activity, we expect that it contains at least some of at least one of the active compounds. Therefore, we figure that DA-19 is worthy of further purification. But, since the MABA activity of DA-19 is appreciably lower than that of DA-1, we figure that other CCC fractions close to #65 should be tested.

We did the same thing with another crude sw/usa residue, DA-17. One of its CCC fractions (fr. #19), DA-3, showed higher MABA activity than DA-17. How much higher, we don’t know because (of course) we don’t know how high “>50” really was (for DA-17) and hence we don’t know how low its activity really was. As above, we expect that DA-3 contains at least one of the active compounds, and that it is also worthy of further purification.

Next, we plan to do CCC on crude residue, DA-12. Since it has higher MABA activity (22.7) than the other two crude residues (above), we expect that one or more of its CCC fractions may have substantial activity.

We also sent crude sw/usa residues to the University of Notre Dame for antibacterial testing. Four had activity against one or more of the bacterial species included in this study, as shown in the “Antibacterial Data” table, all of which are worthy of CCC. All samples had values of 256 or >256 for E. coli, E. aerogenes, A. baumannii, and K. pneumoniae, so these bacterial species are not included in the table.

We applied FCC only to a plant sample which was collected here in Illinois, producing fraction P8 which had anti-TB activity (43.1), but none of the other FCC fractions tested, had any anti-TB activity. We figure that P8 is worthy of further purification, such as by CCC.

References

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