A Little LC-NMR using a simple probe

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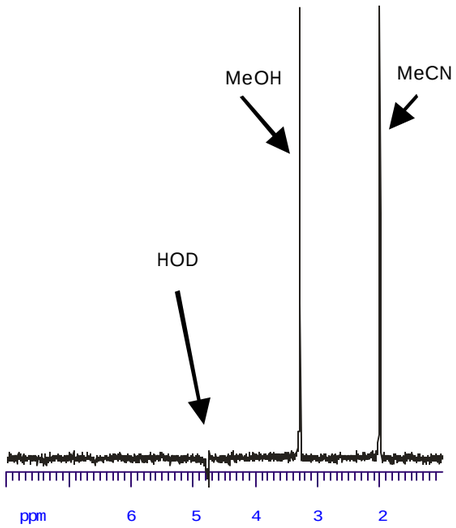
INTRODUCTION

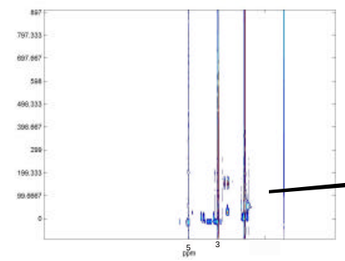
Approximately25%ofwomenwillhaveatleastoneurinarytractinfectionintheirlifetimecaused byinfectionbybacteriaespecially *Escherichiacoli*. Clinicaltrialshave shownthatcranberryjuice preventsandclearsoratleastalleviatestheseinfectionsbyinhibitionofadhesionof*E. coli* to theurinarytractinwomen. *Invitro* experiments haverevealedthatcranberryfruitjuice (*Vacciniummacrocarpon* Aiton,familyEricaceae)possessesanantimicrobialandan antiadhesiveeffect on *E.coli*.Sincelackofsensitivitytowardscommonantibioticsincreasingly complicates treatmentofinfectionsantiadhesiontherapymightbecomeanimportantalternative tothepresentlyusedmethods.Notmuchattentionhasbeenpaidtothehydrophilicfractionof cranberryjuice,maybebecauseofdifficultiesinperformingsuchanalyses.Theappearanceof reversephase columnmaterialsapplicableforseparationofveryhydrophiliccompounds have enabledtheevelopmentofthefast methodforanalysesofthehydrophiliccarboxylicacids presentedhere.HyphenatedmethodslikeHPLC-MSandHPLC-NMR havefacilitated unequivocalstructuraldeterminationofthedetected compounds.

PRACTICALSTUFF.

LC-NMR. AWaterspump,aRheodyne7125injectorvalvewitha100 µl injectionloop,anda ShimadzuSPD-10AUV-VISDetectorwasusedas fortheLC-system. Dataacquisitionand manipulationwereperformedon a C-R8AChromatopac,Shimadzu,Japan.Separationwas accomplished atroomtemperatureonanAquaLUNAC-18(5m;150 4.6mmi.d.)columnfrom Phenomenex. Mobilephase50 µL TFAwasaddedto250mLD2O. ABrukerAMX400WBwithaBrukerflowprobe(120 µL)withaselective ^{1}Handadeuteriumlock channelwasusedastheNMRdetector. WesincerelythankBrukerforlendingustheprobe. Thespectrometerisatwo-channelverionfromabout'89/90allowingonlypresaturation techniquesfor solventsuppressionandonlyonesignalatmost.The1DNOESY-presat was usedforsolventsuppression.



SuppressionoftheresidualHOD signalfromthemobilephase. Thespectrumwasacquiredfrom theeluentafterleavingthecolumn. A1DNOESY-presatsequencewas used. Itisimmediatelyobvious,thatthis columnpreviouslyhasbeenused withmethanolandacetonitrile.



Bothon-flowandstoppedflowtechniqueswasutilized. ForstoppedflowexperimentsaVicimodelEHMAvalvefromValcowasusedtoswitchtheflow toorfromthemagnet.Astopwatchwasusedfortimingtheflowandswitching.

LC-MS. Anisocraticmodewith0.1%(v/v)aqueousformicacidaseluentwasused.Thesame coloumnsfortheUV-detectionwasused,injectionvolumewas50 µl,andtheflowratewas0.5 mL/min,detectionmodeAPI-ESnegative. SamplePreparation.Frozenberrieswerethawedat5Covernightand1partofberriesblended with0.7partsofdeionizedwaterforfiveminutesinaWaringCommercialBlendor.Thepulpwas centrifugedat420gfor15mininaSIGMA3centrifuge.Thesupernatantwasfilteredtogivethe hydrophilic partsofjuice.

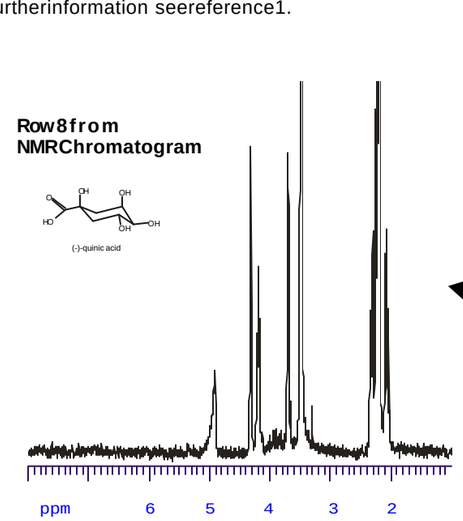
STRUCTURALELUCIDATION

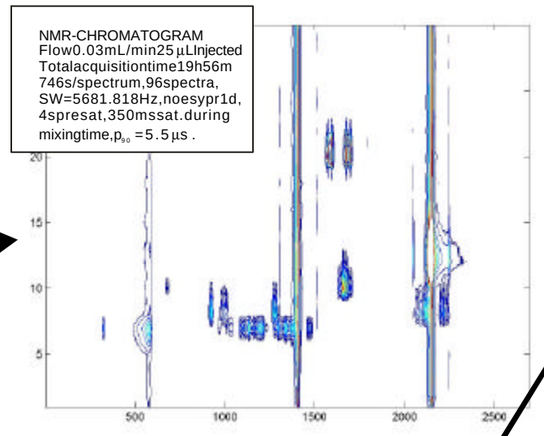
Thecarboxylic acidsquinicacid(peak2),malicacid(peak3),shikimicacid(peak4),andcitric acid(peak5)hadpreviouslybeenstatedtobepresentinthejuice,butnoverificationofthe structureshadbeengiven.HPLC-NMRandHPLC-MSestablishedthestructures.Inadditionthe fourcarboxylic acidswereisolatedbypreparativeHPLCandcomparisonoftheir ^{1}H-NMR spectra

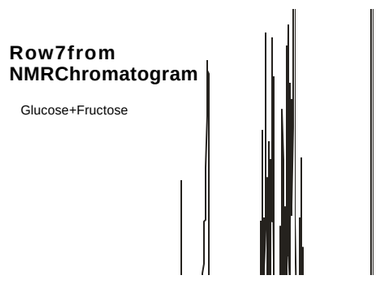
2.08 and1.63ppminthe ^{1}HNMRspectrumandtwosignalsat30.1and35.7ppminthe ^{13}C NMRspectrum.MSrevealedthatthemolecularweightofthecompoundwastwounitshigher than thatofmonotropein.Consequentlythecompoundwasconcludedtobe6,7-dihydromonotropein.

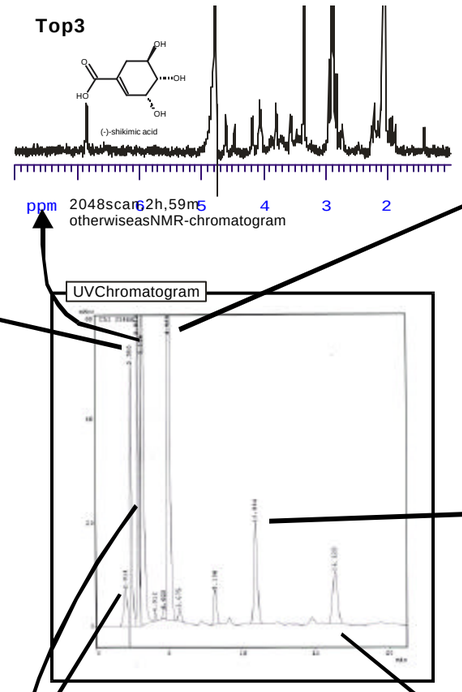
CONCLUSION

LC-NMRincombinationwithLC-MSprovedtobeausefultoolintheanalysisofthehydrophilic fractionofcranberryjuice.Carefulattentiontoexperimentaldetailsandchoiceofproblems allowedtheanalysistobeperformedevenonasimpleflowprobeonaninstrumentthatallows suppressionofonlyonesolventsignal. Forfurtherinformation seereference1.

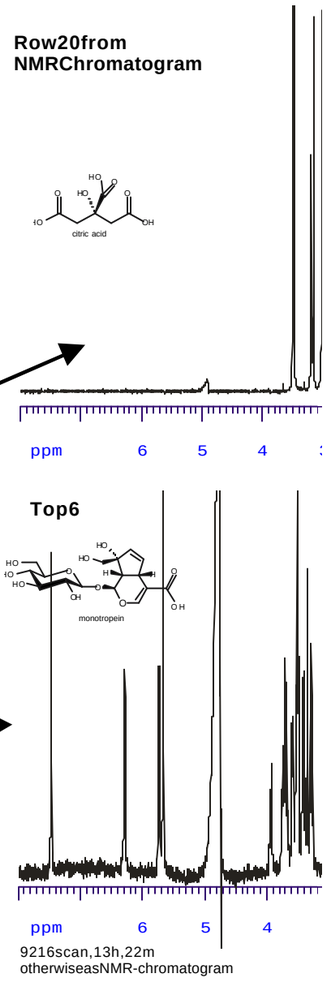


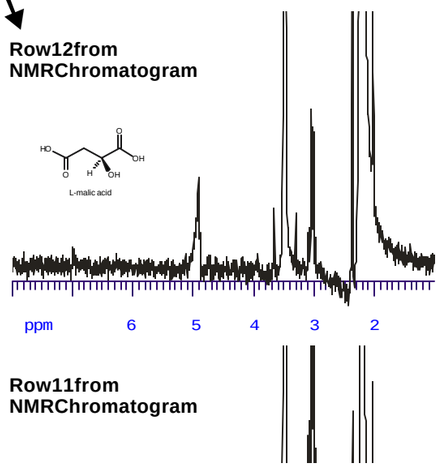


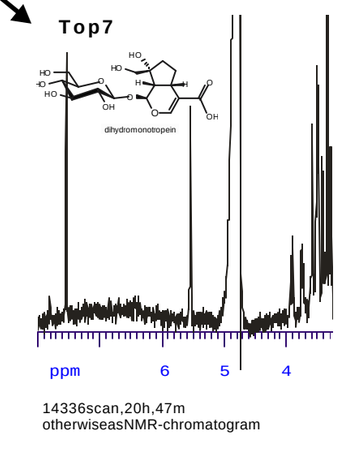




Chromatogramofthehydrophilicfractionofcranberryjuice (*Vacciniummacrocarpon*).Peakat2.0min.amixtureofglucose andfructose,2.4min.quinicacid,2.9min.malicacid, 3.2min.shikimicacid,5.0min.citricacid,10.9min.monotropein, 16.3min.6,7-Dihydromonotropein.







14336scan,20h,47m
otherwiseasNMR-chromatogram