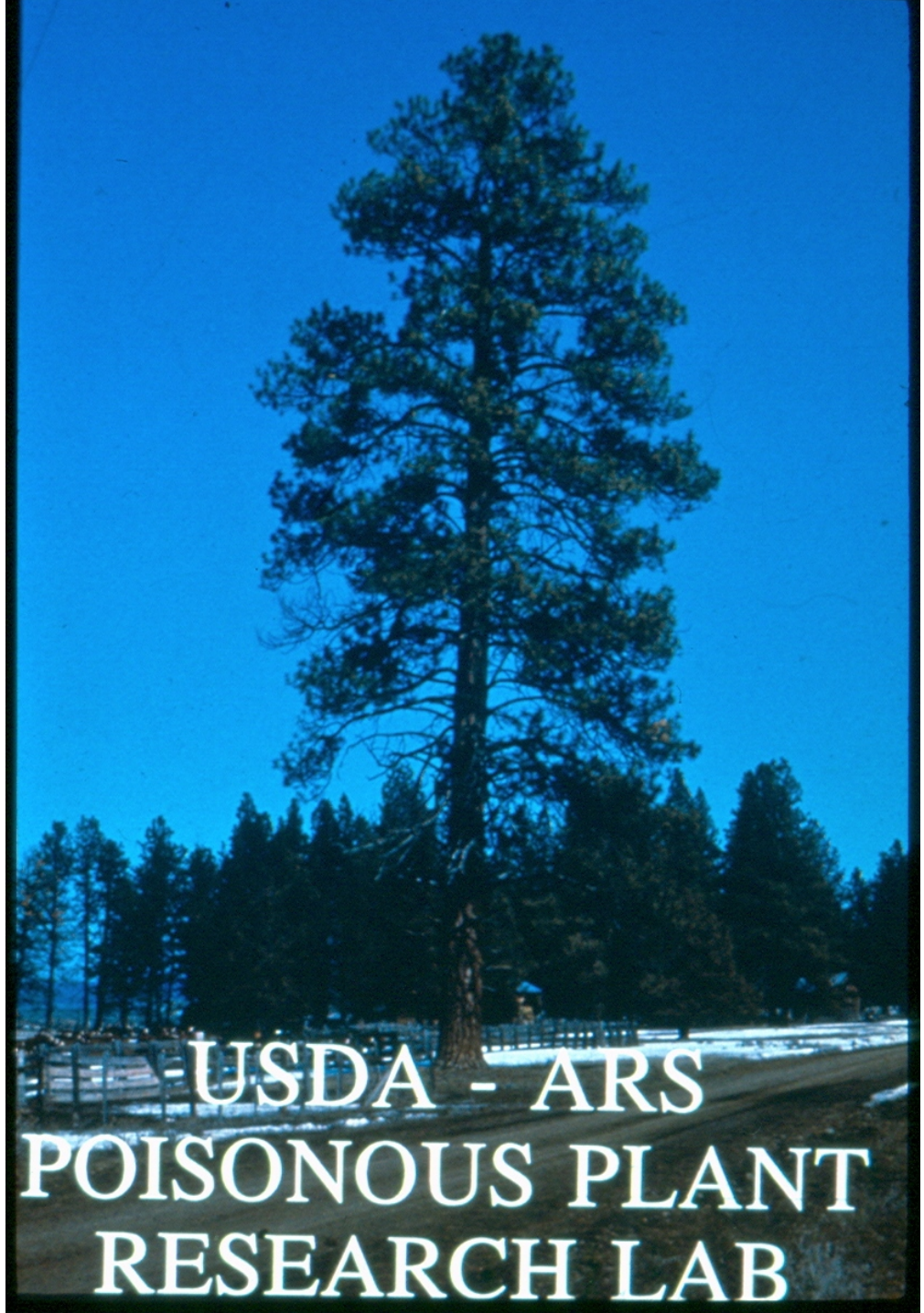
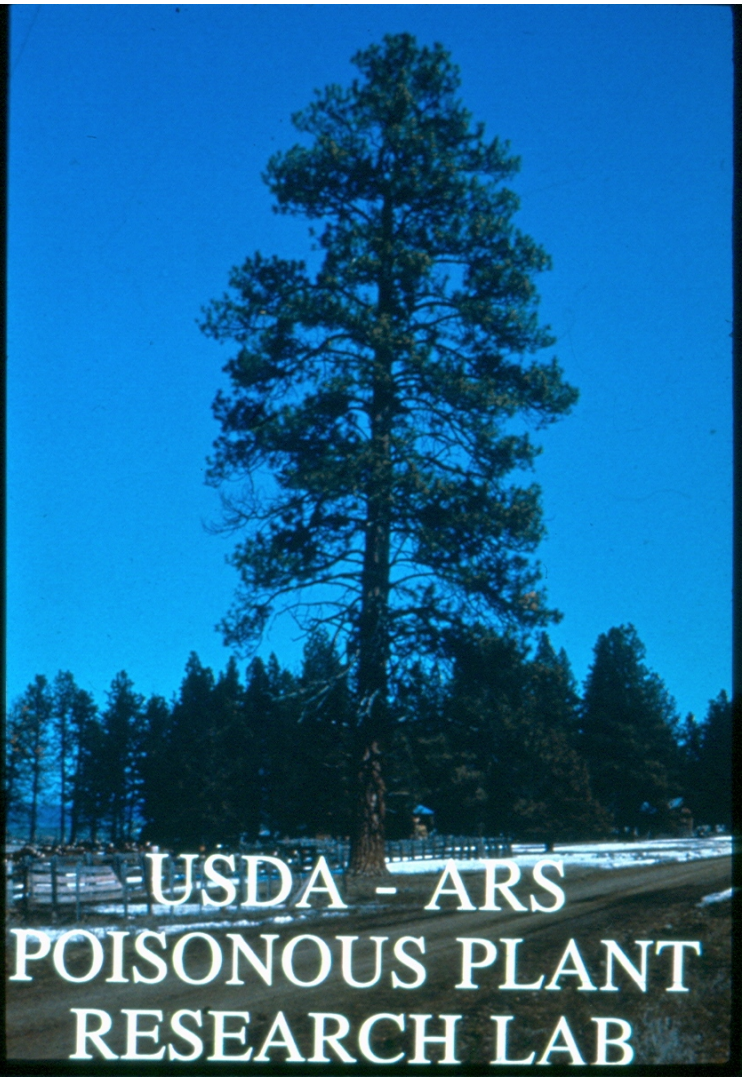
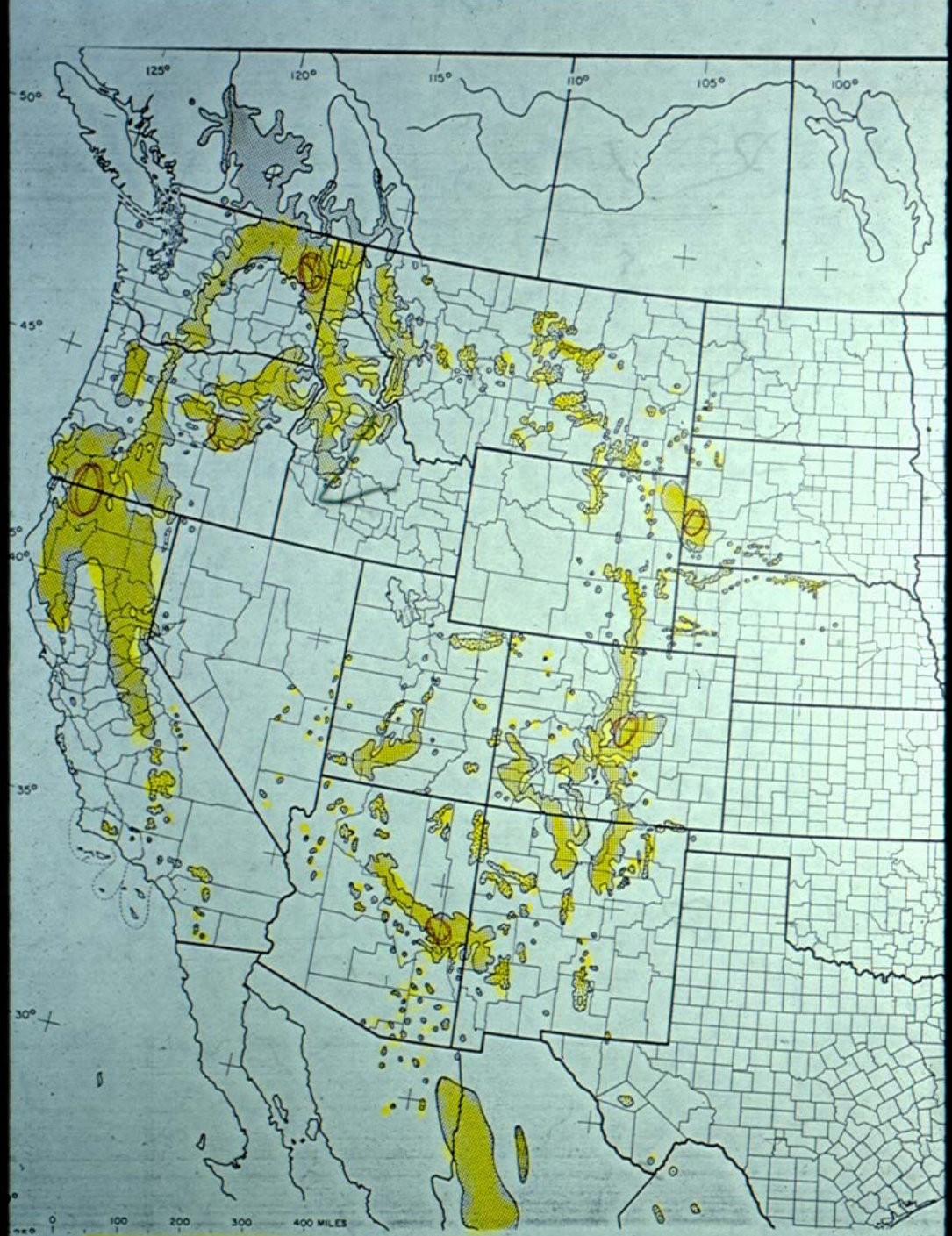

USDA - ARS
POISONOUS PLANT
RESEARCH LAB



var. *scopulorum* (2-3 needles)

var. *ponderosa* (3 needles)

var. *arizonica* (4-5 needles)



Ponderosa pine, Pinus ponderosa

Timber
Forage and cover
Watershed protection
Horticulture and lanscape use
Recreation

Cows will eat pine needles

Fresh green needles

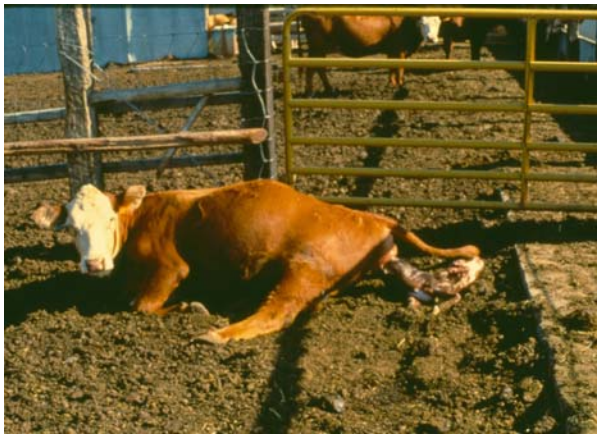
Needles from windfall

Dry older needles (litter) on the ground

Weather changes
Hunger
Changes in Feed
Access to pine needles
Boredom
Accidental ingestion

Abortion may occur within 48 hrs, but usually occur between 6-10 days.

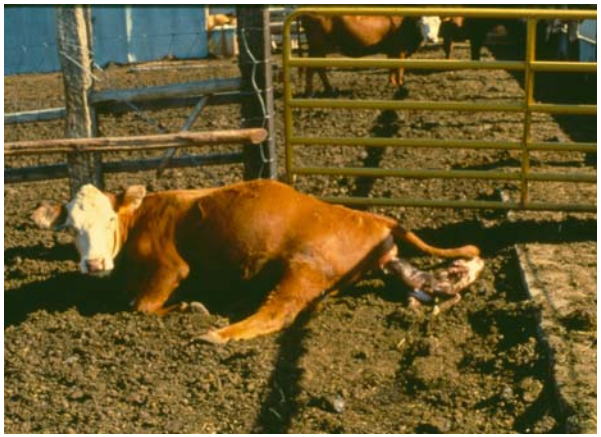
Weak uterine contractions, incomplete cervical dilation and uterine hemorrhage.



Abortion may occur within 48 hrs, but usually occur between 6-10 days.

Weak uterine contractions, incomplete cervical dilation and uterine hemorrhage.

Abortions include stillbirths or the birth of small weak calves.

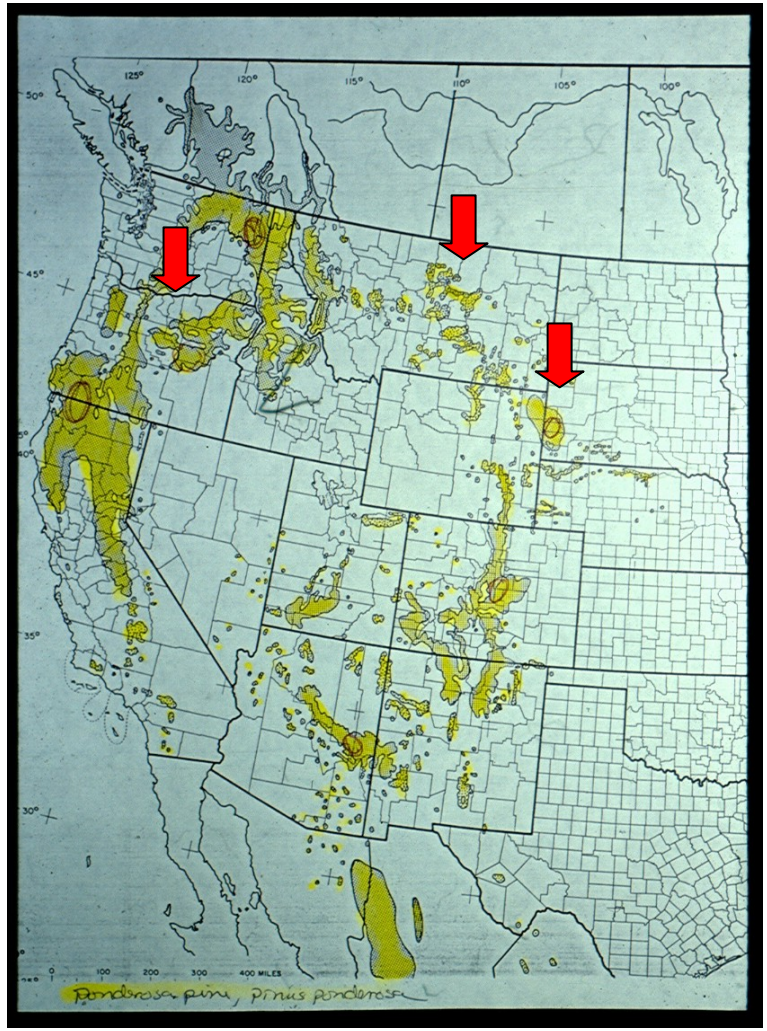


Abortion may occur within 48 hrs, but usually occur between 6-10 days.

Weak uterine contractions, incomplete cervical dilation and uterine hemorrhage.

Abortions include stillbirths or the birth of small weak calves.

Postpartum complications include retained placentas, uterine infection, agalactia, rumen stasis and sometimes death.



Pine Needle Abortion Losses = $5 - $20 million/annually

Bio-assay Guided Fractionation of Pine Needle Abortifacients

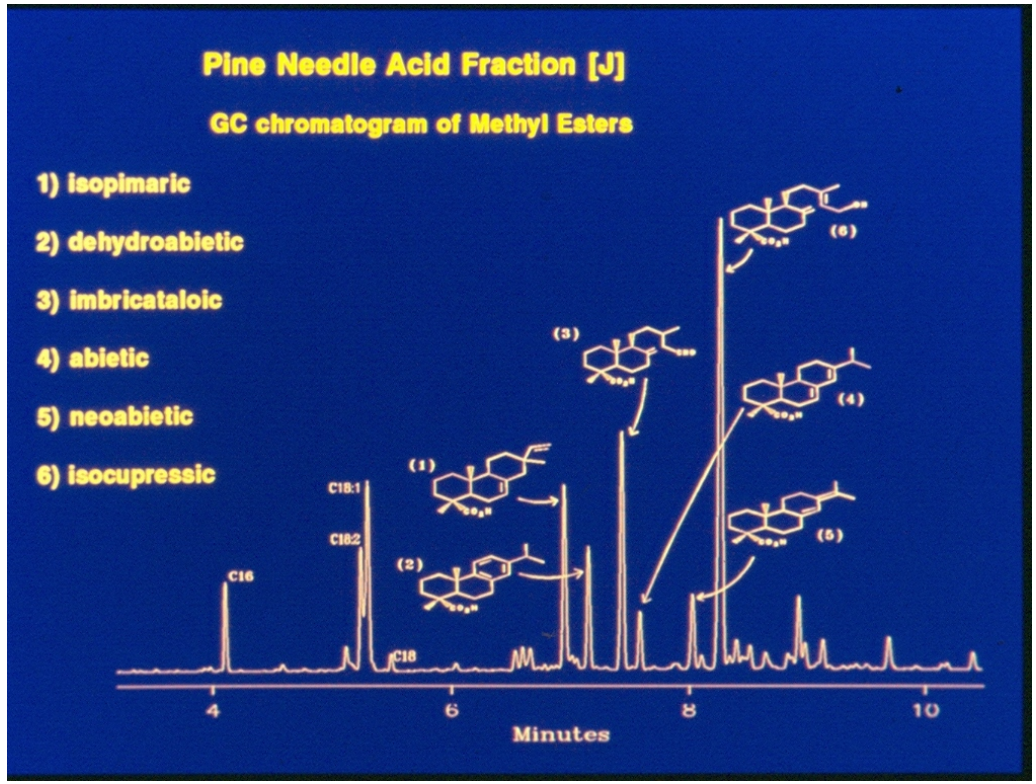
Allen & Kitts, 1961	mice	aqeous fraction
Allison & Kitts, 1964	rats	anti-estrogenic activity
Chow, et al., 1972	mice	water soluble
Cogswell, 1974	mice	water & acetone
Anderson & Lozano, 1977, 1978	mice	aqueous, MeOH, EtOH, $CHCl_3$, hexane
Dehaan, 1979	chick embryo	water/carbohydrates
Kubik & Jackson, 1981	mice	diterpene acids
Shue, 1983	mice	prostaglandins
Gardner, et al., 1994	cows	isocupressic acid
Al-Mahamound, 1995	*in vitro* placentome	dialkyl diols

FLAMMABLE
KEEP FIRE AWA

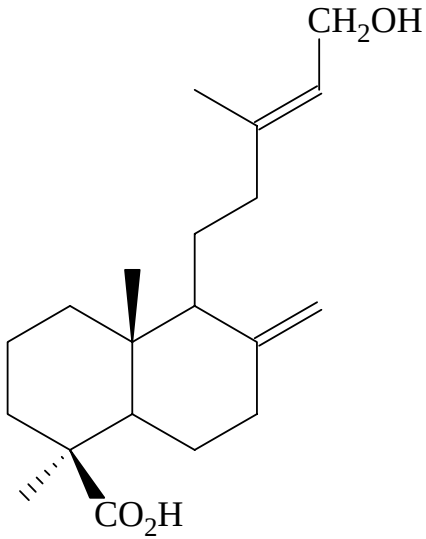
SAFETY FIRST
BE
SAFETY FIRST
CAREFUL
A CAREFUL WORKMAN
IS THE BEST
SAFETY DEVICE

Feeding Trials with Pine Needles, Residues and Extracts

Test Material	# Abortions/ # Test Animals
Pine Needles	7/7
Residue	
Water	3/3
Ethanol	2/6
Acetone	0/1
MeCl	0/4
Hexane	1/2
MeCl Extract	4/4

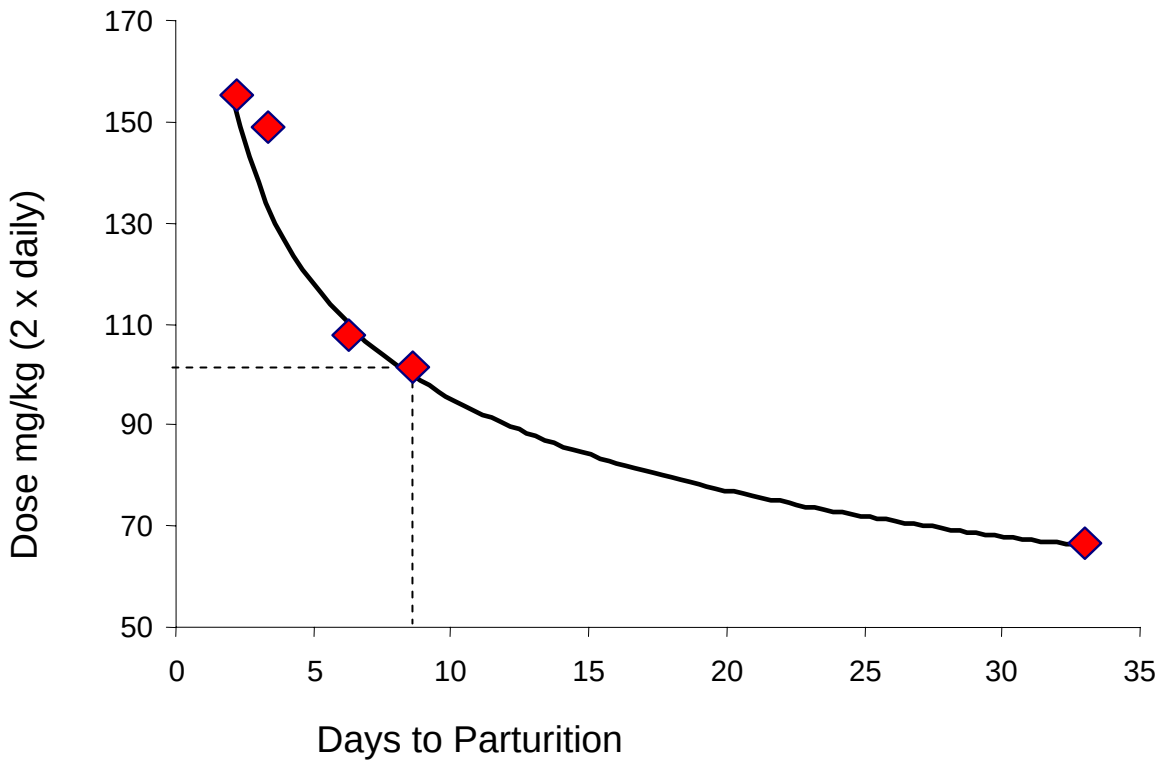

Pine Needle Acid Fraction [J]
GC chromatogram of Methyl Esters
1) isopimaric
2) dehydroabietic
3) imbricataloic
4) abietic
5) neoabietic
6) isocupressic
C16
C18:1
C18:2
C18
(1)
(2)
(3)
(4)
(5)
(6)
4
6
8
10
Minutes

Abortifacient Compound in Ponderosa Pine Needles



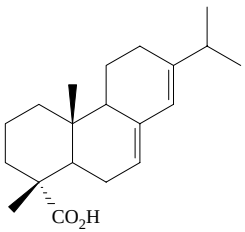
Isocupressic Acid

Feeding Trials with Isocupressic Acid

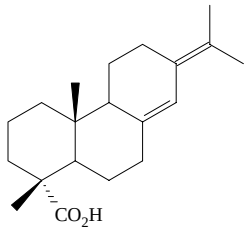


Diterpene Acid of Ponderosa Pine

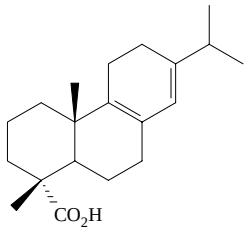
Tricyclic Abietane Acids



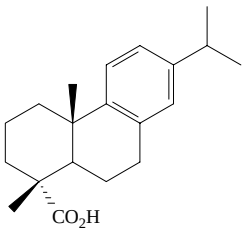
ABIETIC ACID



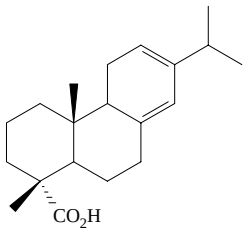
NEOABIETIC ACID



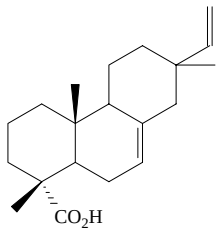
PALUSTRIC ACID



DEHYDROABIETIC ACID

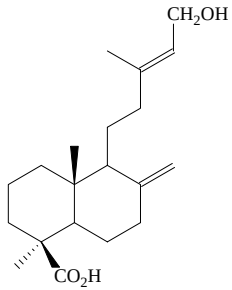


LEVOPIMARIC ACID

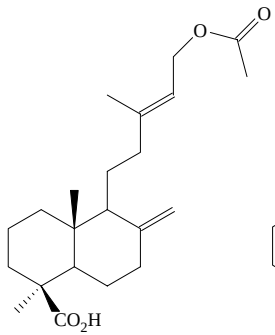


ISOPIMARIC ACID

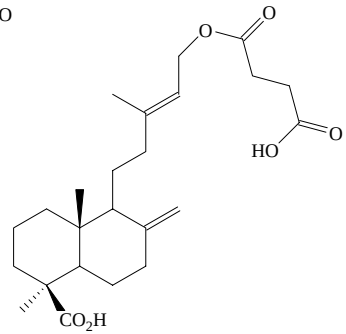
Labdane Acids



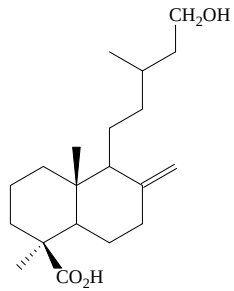
ISOCUPRESSIC ACID



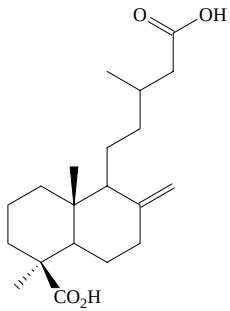
ACETYLISOCUPRESSIC ACID



SUCCINYLISOCUPRESSIC ACID

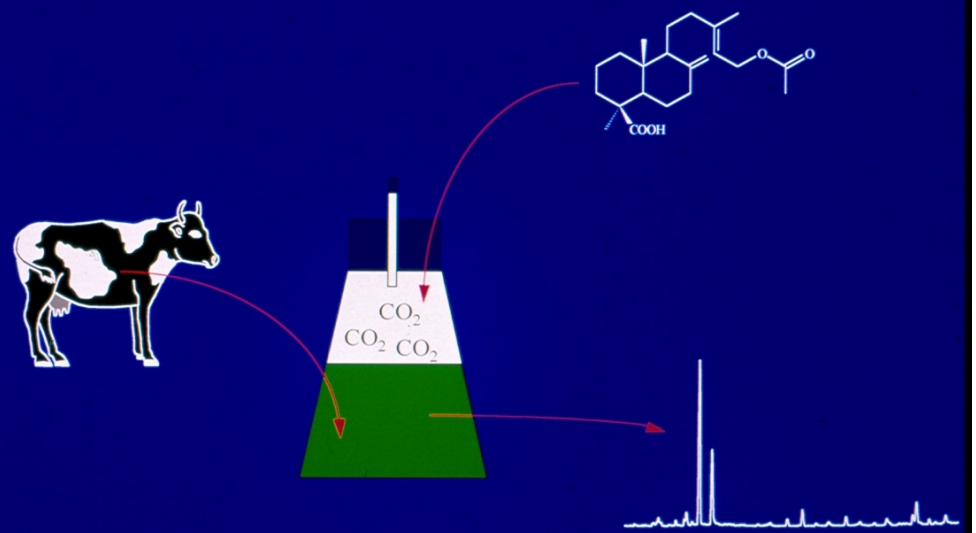


IMBRICATOLOIC ACID

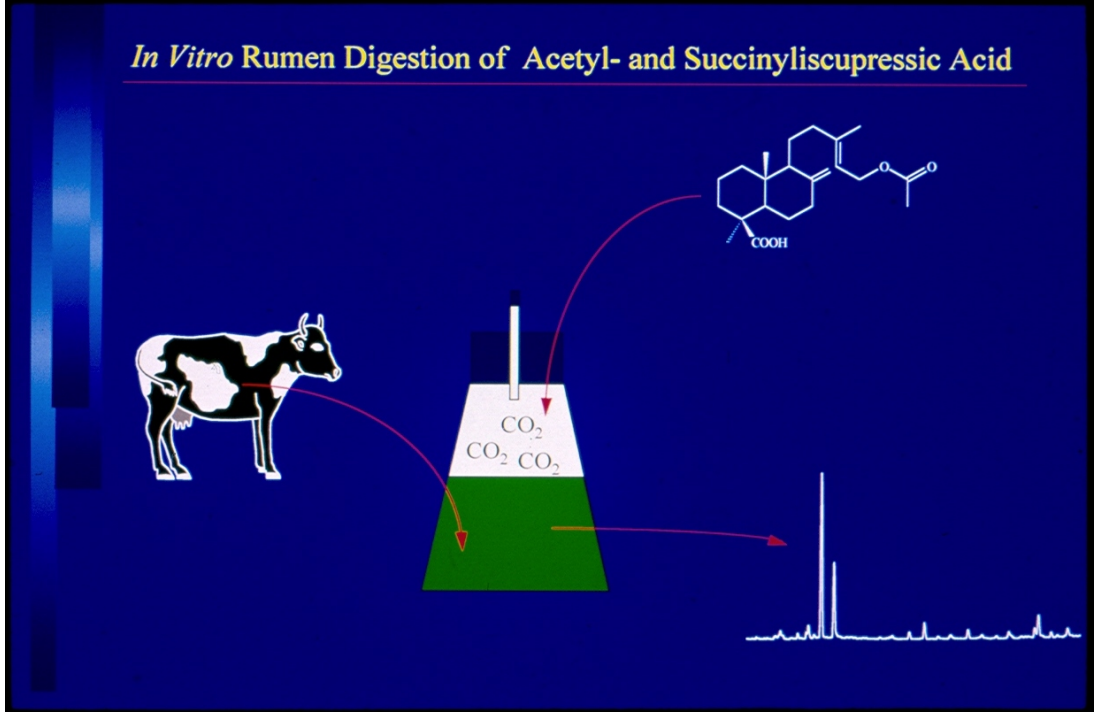


DIHYDROAGATHIC ACID

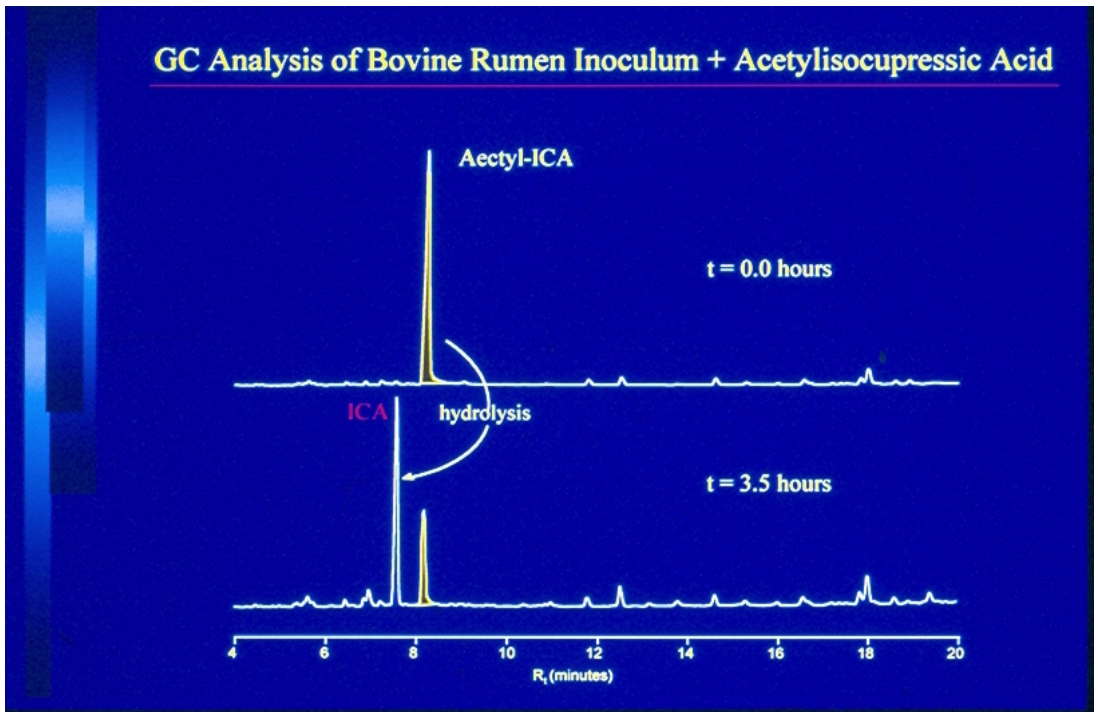
In Vitro Rumen Digestion of Acetyl- and Succinyliscupressic Acid



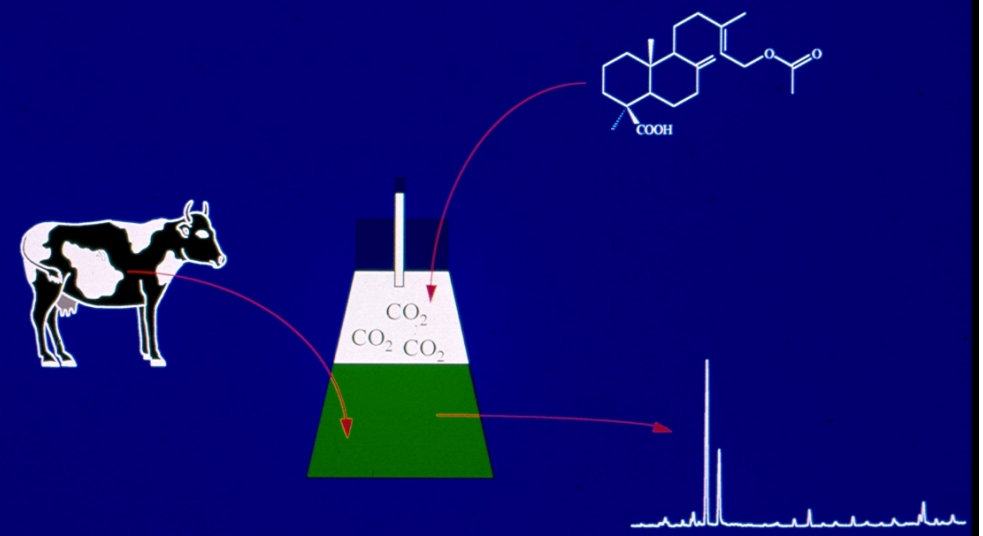
In Vitro Rumen Digestion of Acetyl- and Succinyliscupressic Acid



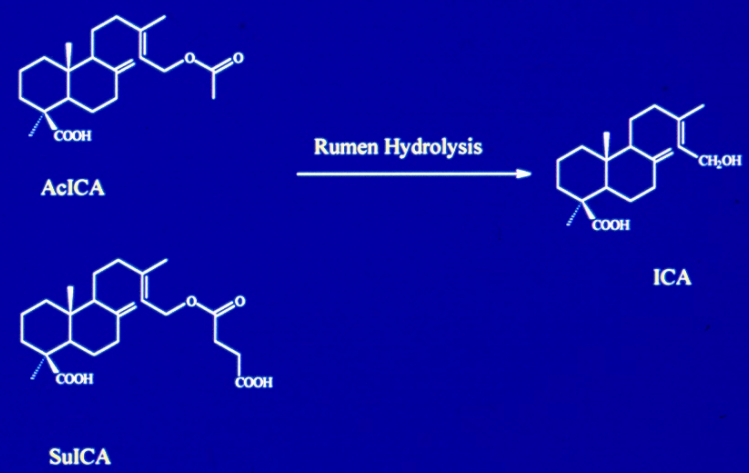
GC Analysis of Bovine Rumen Inoculum + Acetylisocupressic Acid



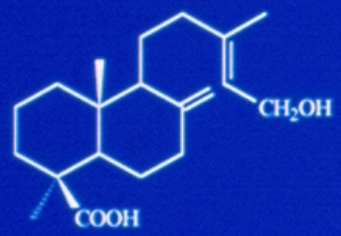
In Vitro Rumen Digestion of Acetyl- and Succinyliscupressic Acid



Ruminal Metabolism of Acetyl- and Succinyl-ICA



Isocupressic Acid Serum Content



ICA

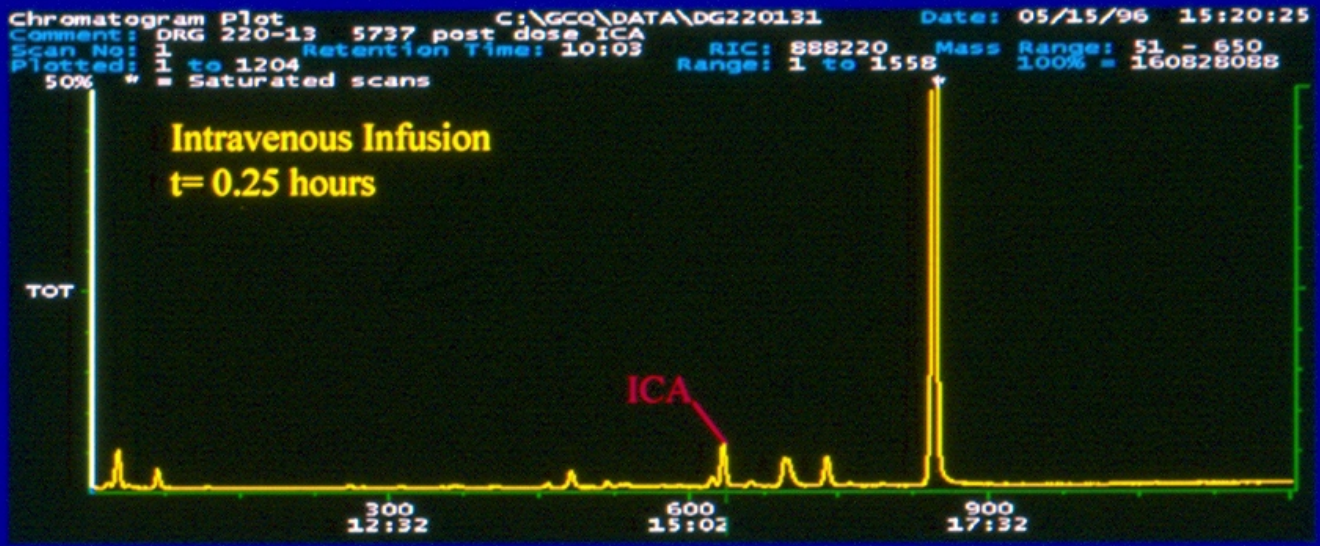
intravenous

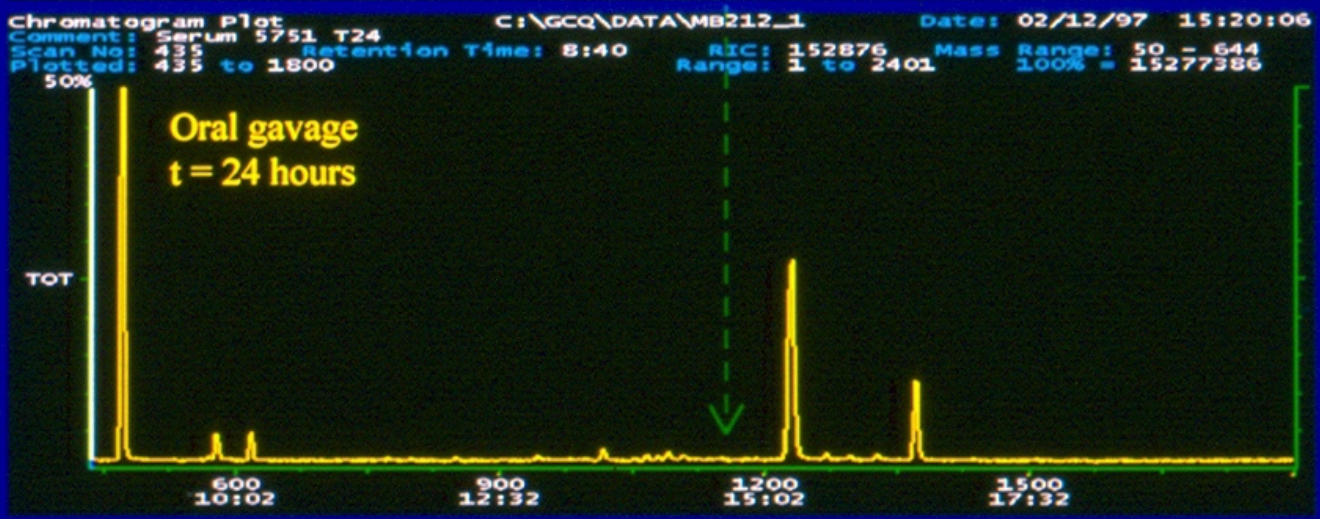
Blood Serum

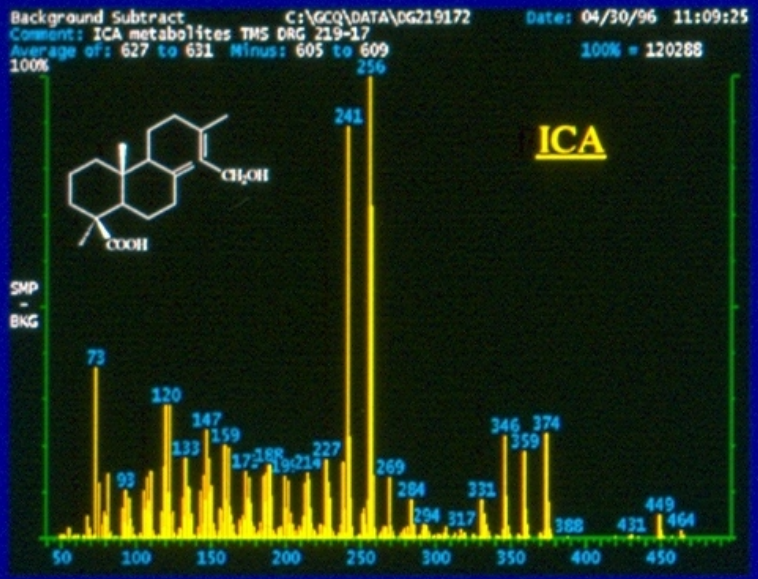
ICA

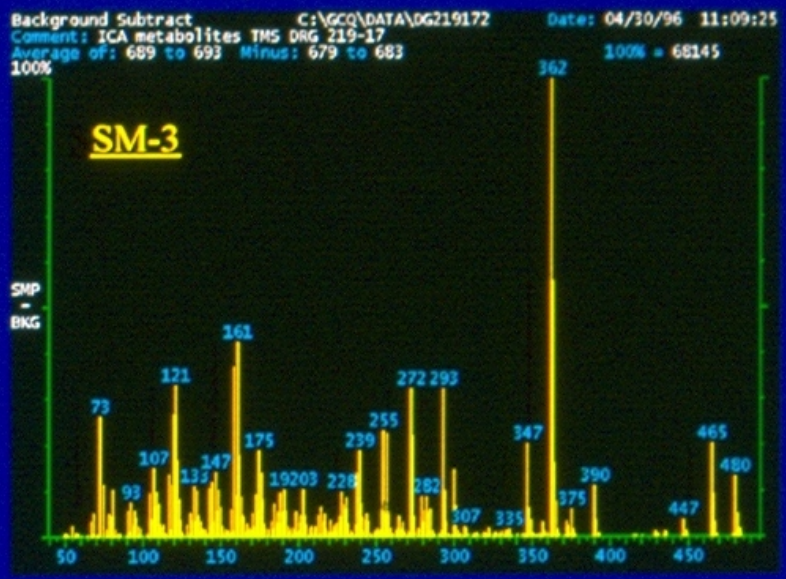
?

Serum ICA Analysis






Background Subtract C:\GCQ\DATA\DG219172 Date: 04/30/96 11:09:25
Comment: ICA metabolites TMS DRG 219-17
Average of: 627 to 631 Minus: 605 to 609
100% = 120288
ICA
CH2OH
COOH
SMP - BKG
73 93 120 133 147 159 227 241 256 269 284 294 317 331 346 359 374 388 431 449 464
50 100 150 200 250 300 350 400 450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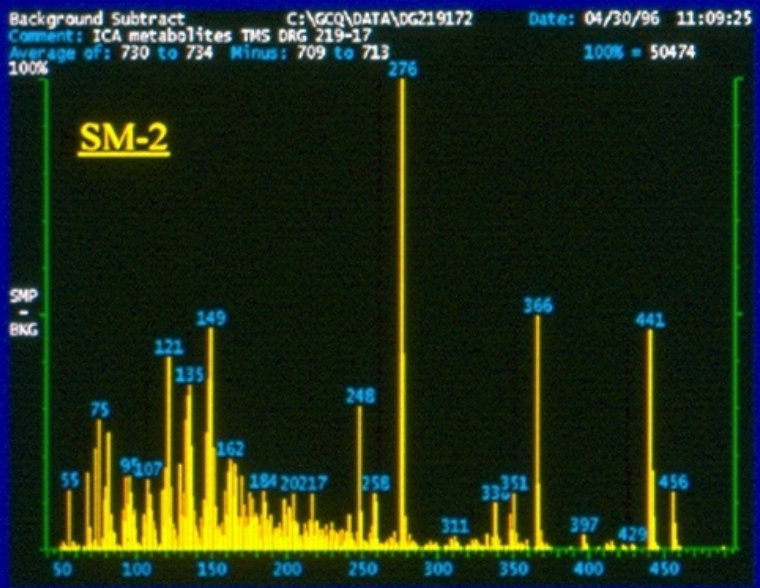
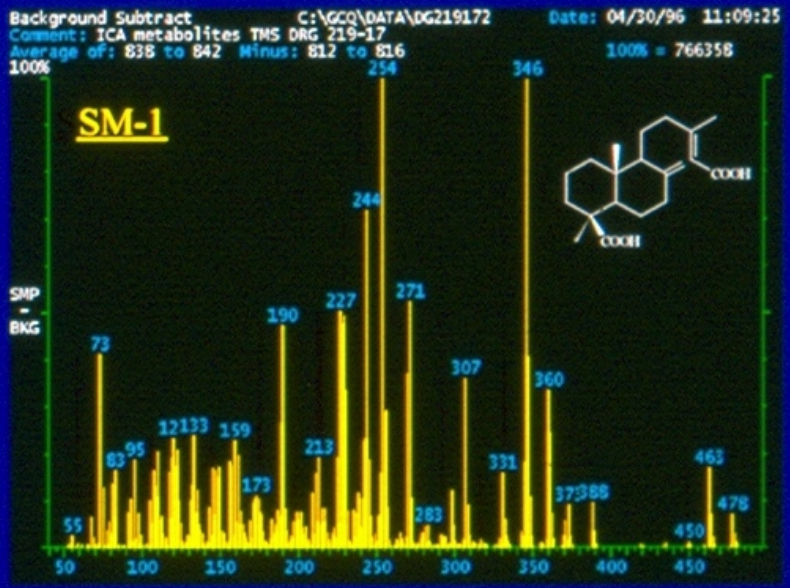
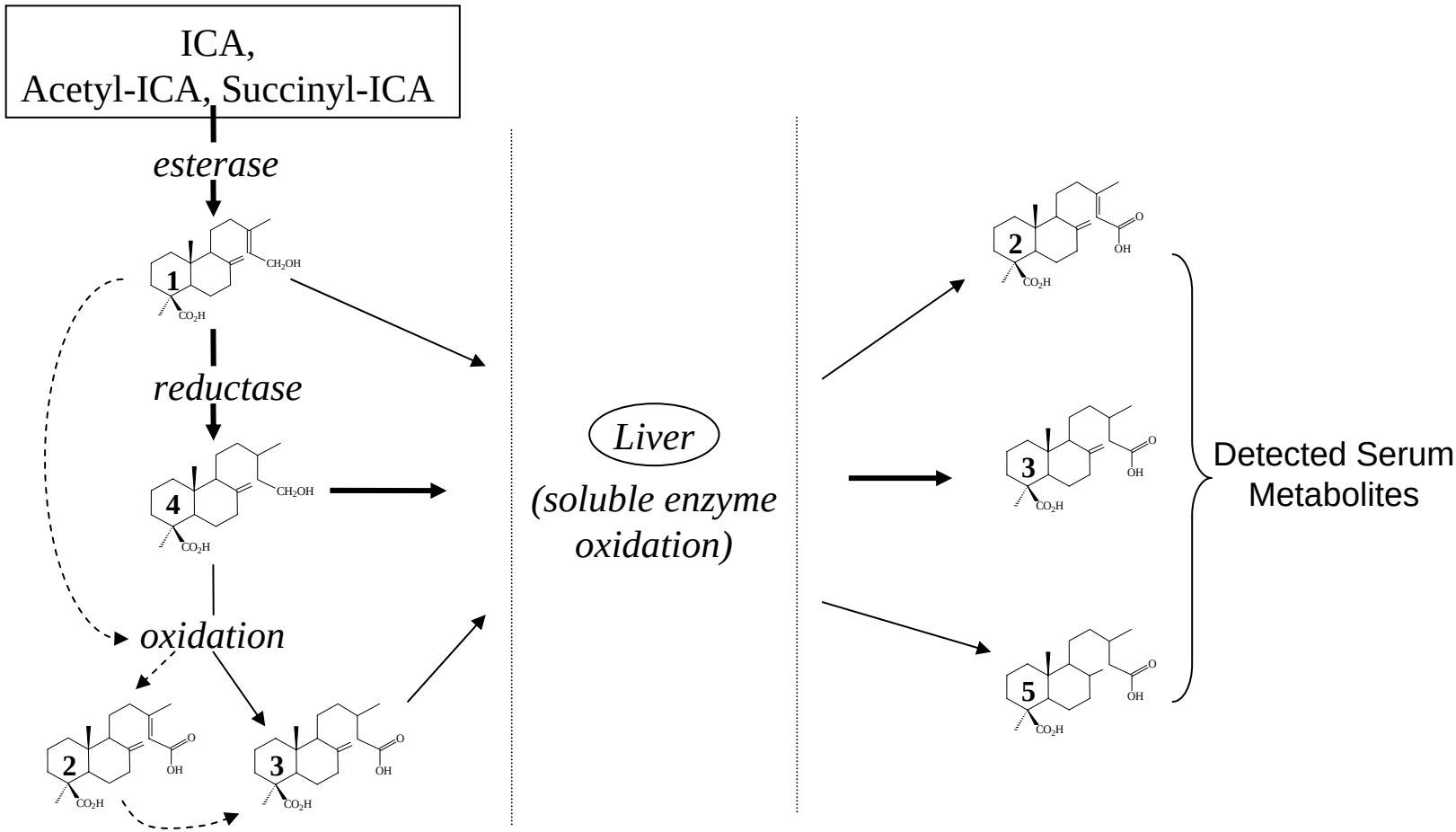
Comment: ICA metabolites TMS DRG 219-17
Average of: 689 to 693 Minus: 679 to 683
100% = 68145
SM-3
SMP - BKG
73 93 107 121 133 147 161 175 19203 228 239 255 272 282 293 307 335 347 362 375 390 447 465 480
50 100 150 200 250 300 350 400 450

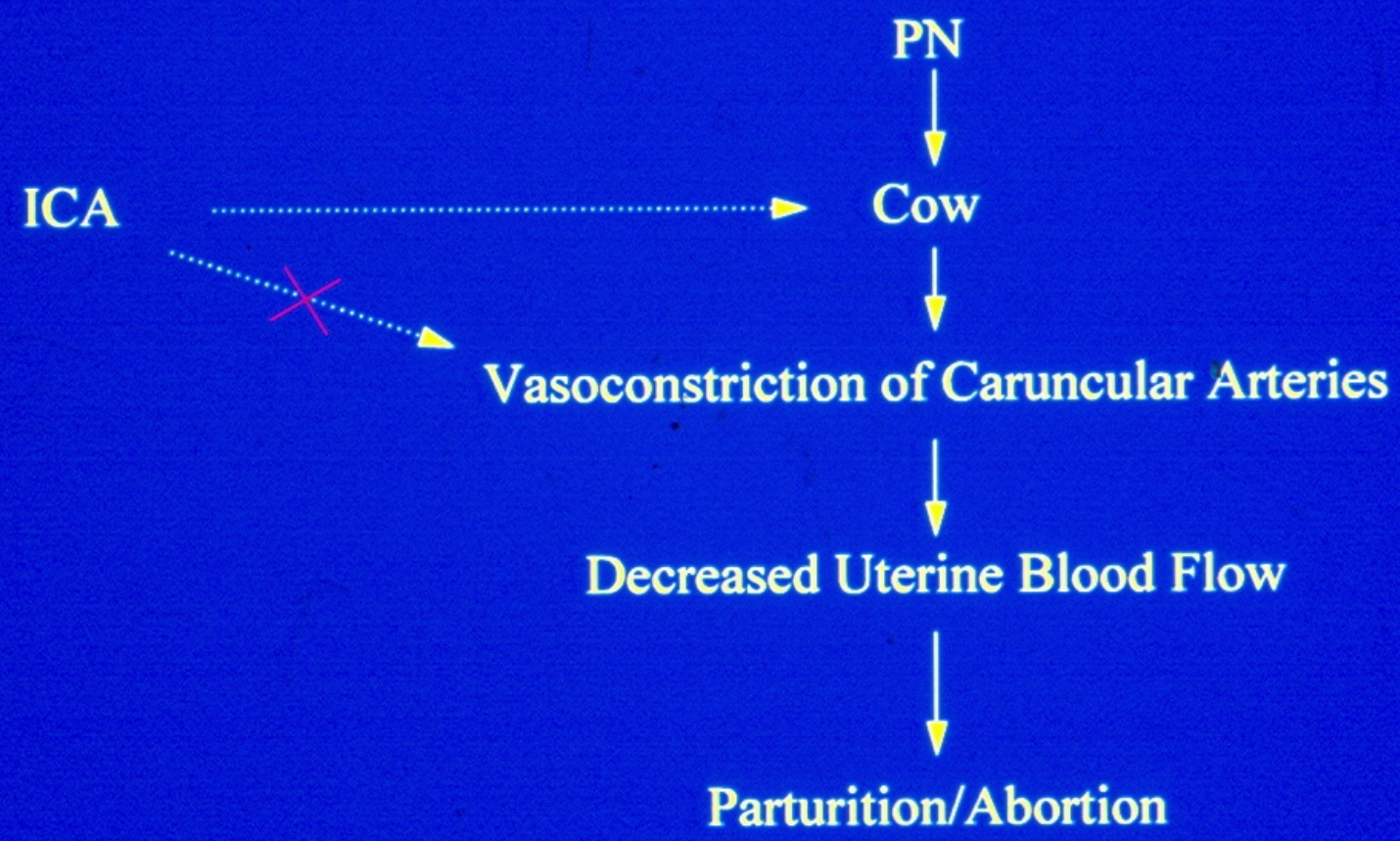

Background Subtract C:\GCQ\DATA\DG219172 Date: 04/30/96 11:09:25
Comment: ICA metabolites TMS DRG 219-17
Average of: 730 to 734 Minus: 709 to 713
100% = 50474
SM-2
SMP - BKG
55 75 95 107 121 135 149 162 184 20217 248 258 276 311 330 351 366 397 429 441 456
50 100 150 200 250 300 350 400 450


Background Subtract C:\GCQ\DATA\DG219172 Date: 04/30/96 11:09:25
Comment: ICA metabolites TMS DRG 219-17
Average of: 838 to 842 Minus: 812 to 816
100% = 766358
SM-1
COOH
COOH
SMP - BKG
55 73 83 95 12133 159 173 190 213 227 244 254 271 283 307 331 346 360 373 388 450 463 478
50 100 150 200 250 300 350 400 450

5. Isocupressic acid **is metabolized** in both the rumen and the hepatic systems



Proposed Abortion Mechanism*



*Ford, et al., *J. Anim. Sci.* **70**:1609-1614 (1992)

Isocupressic Acid Content of Selected Species and Locations

Species	Common Name	Locations	ICA % DW
Pinus ponderosa	Ponderosa Pine	OR, AZ, CA, UT, CO, WY	0.42 – 1.35
Pins contorta	Lodgepole Pine	OR, ID, CO, UT, BC, AR	0.00 – 0.66
Pinus jeffreyi	Jeffrey Pine	CA	0.04 – 0.54
Pinus arizonica	Arizona Pine	CA, AZ	n.d. – 0.49
Pinus edulis	Pinyon Pine	AZ, CO, NM, UT	n.d. – 0.45
Pinus monophylla	White Pine	NV	n.d. – 0.32
Pinus taeda	Loblolly Pine	AR, AZ	n.d. – trace
Pinus flexilis	Limber Pine	CO, UT	n.d. – 0.06
Pinus echinata	Short leaf pine	AR	n.d.
Pinus elliottii	Slash Pine	AR	n.d.
Pinus palustris	Longleaf Pine	AR	n.d.
Pinus montezumae	Montezuma Pine	CC	n.d.
Abies concolor	White Fir	CA, CO, UT	n.d. – 0.04
Abies lasiocarpa	Subalpine Fir	OR, ID	0.01 – 0.04
Pseudotsuga menziesii	Douglas Fir	UT, CA, ID, AZ, OR	n.d. – 0.04
Juniperus communis	Mountain common juniper	CO, UT	2.05 – 4.5
Juniperus scopulorum	Rocky Mt. juniper	UT, NM, AZ	0.25 – 0.84
Juniperus osteosperma	Utah juniper	UT, NV,	n.d. – 0.07
Juniperus californicum	California juniper	CA	0.05 – 1.4
Juniperus monosperma	One Seed Juniper	AZ, NM	n.d. – 0.14
Thuja plicata	Western Red Cedar	AZ, NM, UT	0.33 – 0.84
Pinus koraiensis	Korean Pine	UT, Korea	n.d. – trace
Picea engelmannii	Engelmann Spruce	CA, CO, ID, MT, OR, UT	n.d. – 0.31
Picea pungens	Blue Spruce	UT, CO	n.d. – 0.17
Cupressus macrocarpa	Monterey Cypress	CA, New Zealand	n.d. – 1.24
Pinus longaeva	Bristle cone pine	CO	0.01 – 0.05
Abies grandis	Grand Fir	OR	n.d.
Tsuga mertensiana	Mt. Hemlock	OR	n.d.
Larix occidentalis	Wester Larch	OR	n.d.
Libocedrus decurrens	Incense cedar	OR	0.07
Abies procera	Red fir	CA	0.05

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Pinus arizonica	Arizona Pine	CA, AZ	n.d. – 0.49
Pinus edulis	Pinyon Pine	AZ, CO, NM, UT	n.d. – 0.45
Pinus monophylla	White Pine	NV	n.d. – 0.32
Pinus taeda	Loblolly Pine	AR, AZ	n.d. – trace
Pinus flexilis	Limber Pine	CO, UT	n.d. – 0.06
Pinus echinata	Short leaf pine	AR	n.d.
Pinus elliottii	Slash Pine	AR	n.d.
Pinus palustris	Longleaf Pine	AR	n.d.
Pinus montezumae	Montezuma Pine	CC	n.d.
Abies concolor	White Fir	CA, CO, UT	n.d. – 0.04
Abies lasiocarpa	Subalpine Fir	OR, ID	0.01 – 0.04
Pseudotsuga menziesii	Douglas Fir	UT, CA, ID, AZ, OR	n.d. – 0.04
Juniperus communis	Mountain common juniper	CO, UT	2.05 – 4.5
Juniperus scopulorum	Rocky Mt. juniper	UT, NM, AZ	0.25 – 0.84
Juniperus osteosperma	Utah juniper	UT, NV,	n.d. – 0.07
Juniperus californicum	California juniper	CA	0.05 – 1.4
Juniperus monosperma	One Seed Juniper	AZ, NM	n.d. – 0.14
Thuja plicata	Western Red Cedar	AZ, NM, UT	0.33 – 0.84
Pinus koraiensis	Korean Pine	UT, Korea	n.d. – trace
Picea engelmannii	Engelmann Spruce	CA, CO, ID, MT, OR, UT	n.d. – 0.31
Picea pungens	Blue Spruce	UT, CO	n.d. – 0.17
Cupressus macrocarpa	**Monterey Cypress**	**CA, New Zealand**	**n.d. – 1.24**
Pinus longaeva	Bristle cone pine	CO	0.01 – 0.05
Abies grandis	Grand Fir	OR	n.d.
Tsuga mertensiana	Mt. Hemlock	OR	n.d.
Larix occidentalis	Wester Larch	OR	n.d.
Libocedrus decurrens	Incense cedar	OR	0.07
Abies procera	Red fir	CA	0.05

Isocupressic Acid Content of Selected Species and Locations

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Pinus ponderosa	Ponderosa Pine	OR, AZ, CA, UT, CO, WY	0.42 – 1.35
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Pinus arizonica	Arizona Pine	CA, AZ	n.d. – 0.49
Pinus edulis	Pinyon Pine	AZ, CO, NM, UT	n.d. – 0.45
Pinus monophylla	White Pine	NV	n.d. – 0.32
Pinus taeda	Loblolly Pine	AR, AZ	n.d. – trace
Pinus flexilis	Limber Pine	CO, UT	n.d. – 0.06
Pinus echinata	Short leaf pine	AR	n.d.
Pinus elliottii	Slash Pine	AR	n.d.
Pinus palustris	Longleaf Pine	AR	n.d.
Pinus montezumae	Montezuma Pine	CC	n.d.
Abies concolor	White Fir	CA, CO, UT	n.d. – 0.04
Abies lasiocarpa	Subalpine Fir	OR, ID	0.01 – 0.04
Pseudotsuga menziesii	Douglas Fir	UT, CA, ID, AZ, OR	n.d. – 0.04
Juniperus communis	Mountain common juniper	CO, UT	2.05 – 4.5
Juniperus scopulorum	Rocky Mt. juniper	UT, NM, AZ	0.25 – 0.84
Juniperus osteosperma	Utah juniper	UT, NV,	n.d. – 0.07
Juniperus californicum	California juniper	CA	0.05 – 1.4
Juniperus monosperma	One Seed Juniper	AZ, NM	n.d. – 0.14
Thuja plicata	Western Red Cedar	AZ, NM, UT	0.33 – 0.84
Pinus koraiensis	Korean Pine	UT, Korea	n.d. – trace
Picea engelmannii	Engelmann Spruce	CA, CO, ID, MT, OR, UT	n.d. – 0.31
Picea pungens	Blue Spruce	UT, CO	n.d. – 0.17
Cupressus macrocarpa	Monterey Cypress	CA, New Zealand	n.d. – 1.24
Pinus longaeva	Bristle cone pine	CO	0.01 – 0.05
Abies grandis	Grand Fir	OR	n.d.
Tsuga mertensiana	Mt. Hemlock	OR	n.d.
Larix occidentalis	Wester Larch	OR	n.d.
Libocedrus decurrens	Incense cedar	OR	0.07
Abies procera	Red fir	CA	0.05

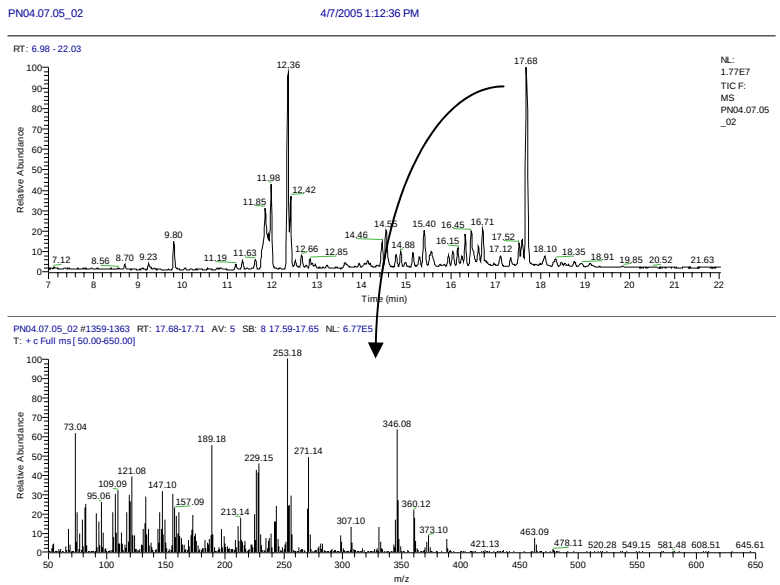
Isocupressic Acid Content of Selected Species and Locations

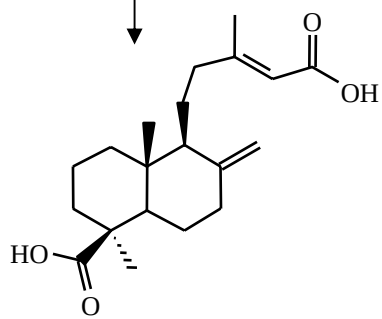
Species	Common Name	Locations	ICA % DW
Pinus ponderosa	Ponderosa Pine	OR, AZ, CA, UT, CO, WY	0.42 – 1.35
Pins contorta	Lodgepole Pine	OR, ID, CO, UT, BC, AR	0.00 – 0.66
Pinus jeffreyi	Jeffrey Pine	CA	0.04 – 0.54
Pinus arizonica	Arizona Pine	CA, AZ	n.d. – 0.49
Pinus edulis	Pinyon Pine	AZ, CO, NM, UT	n.d. – 0.45
Pinus monophylla	White Pine	NV	n.d. – 0.32
Pinus taeda	Loblolly Pine	AR, AZ	n.d. – trace
Pinus flexilis	Limber Pine	CO, UT	n.d. – 0.06
Pinus echinata	Short leaf pine	AR	n.d.
Pinus elliottii	Slash Pine	AR	n.d.
Pinus palustris	Longleaf Pine	AR	n.d.
Pinus montezumae	Montezuma Pine	CC	n.d.
Abies concolor	White Fir	CA, CO, UT	n.d. – 0.04
Abies lasiocarpa	Subalpine Fir	OR, ID	0.01 – 0.04
Pseudotsuga menziesii	Douglas Fir	UT, CA, ID, AZ, OR	n.d. – 0.04
Juniperus communis	**Mountain common juniper**	**CO, UT**	**2.05 – 4.5**
Juniperus scopulorum	Rocky Mt. juniper	UT, NM, AZ	0.25 – 0.84
Juniperus osteosperma	Utah juniper	UT, NV,	n.d. – 0.07
Juniperus californicum	California juniper	CA	0.05 – 1.4
Juniperus monosperma	One Seed Juniper	AZ, NM	n.d. – 0.14
Thuja plicata	Western Red Cedar	AZ, NM, UT	0.33 – 0.84
Pinus koraiensis	Korean Pine	UT, Korea	n.d. – trace
Picea engelmannii	Engelmann Spruce	CA, CO, ID, MT, OR, UT	n.d. – 0.31
Picea pungens	Blue Spruce	UT, CO	n.d. – 0.17
Cupressus macrocarpa	Monterey Cypress	CA, New Zealand	n.d. – 1.24
Pinus longaeva	Bristle cone pine	CO	0.01 – 0.05
Abies grandis	Grand Fir	OR	n.d.
Tsuga mertensiana	Mt. Hemlock	OR	n.d.
Larix occidentalis	Wester Larch	OR	n.d.
Libocedrus decurrens	Incense cedar	OR	0.07
Abies procera	Red fir	CA	0.05

Utah Juniper

Juniperus osteosperma

Utah Juniper Bark





Agathic acid ~ 1% dry weight

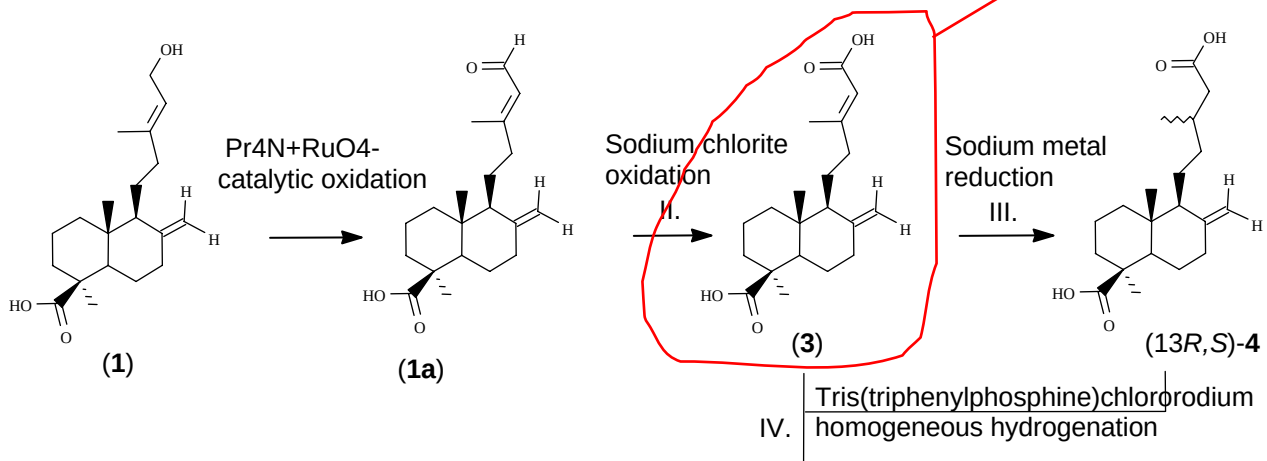
Bioassay for Utah Juniper Bark

Asssay	Dose	# Abortions/ #Tested	Time
Cows	106 – 143	3/3	4 – 9 days
Sheep	110 – 150	0/5	n.a.

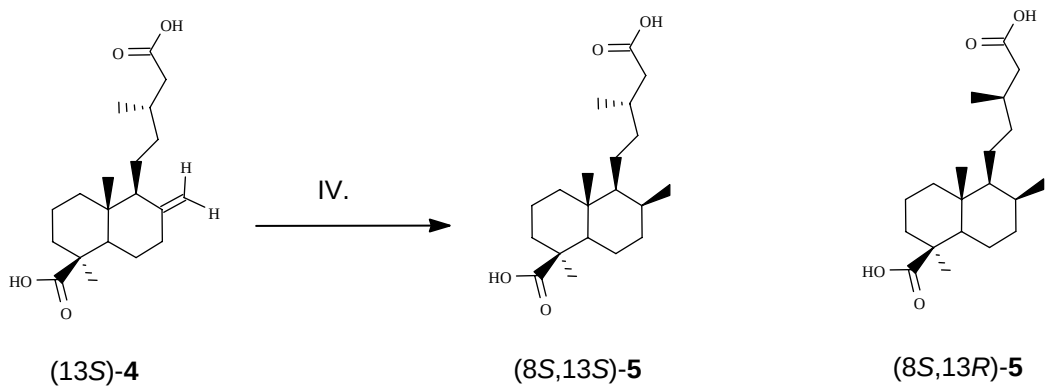
Synthetic Preparation of Isocupressic Acid Metabolites

Yes - Abortifacient

Scheme 1. Conversion of isocupressic acid (**1**) to (13*R*,*S*)-tetrahydroagathic acid (**5**).



Scheme 2. Conversion of (13*S*)-dihydroagathic acid (**4**) to (13*S*)-tetrahydroagathic acid (**5**).



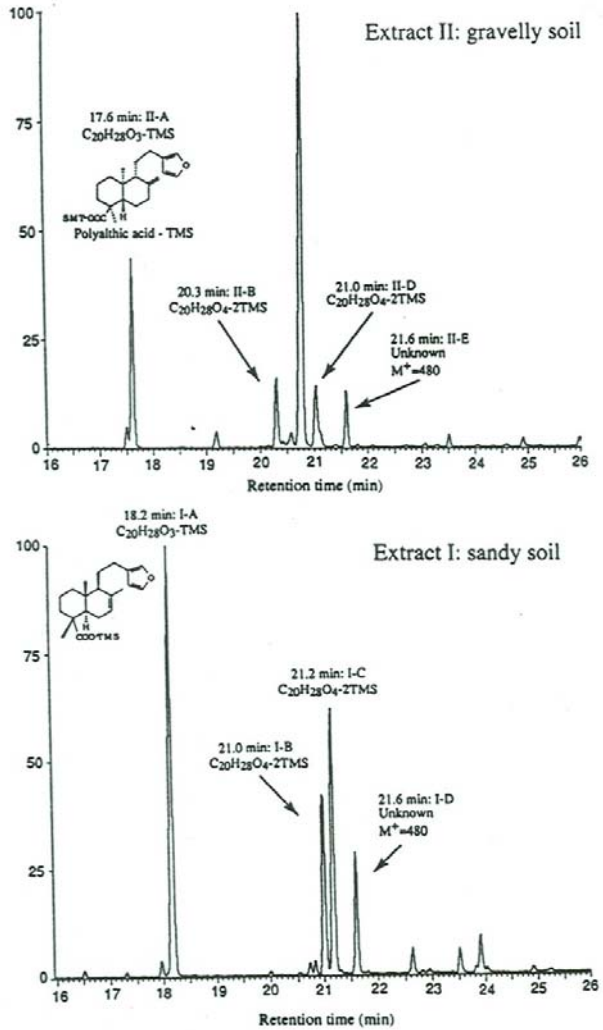
Broom snakeweed (*Gutierrezia sarothrea*)

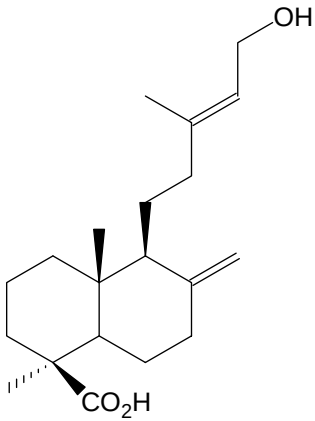
Toxic and abortifacient to cattle

Broom snakeweed (*Gutierrezia sarothrea*)

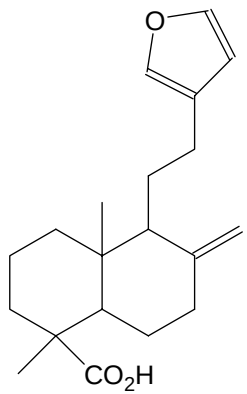
Toxic and abortifacient to cattle

Contains furano-diterpene acids

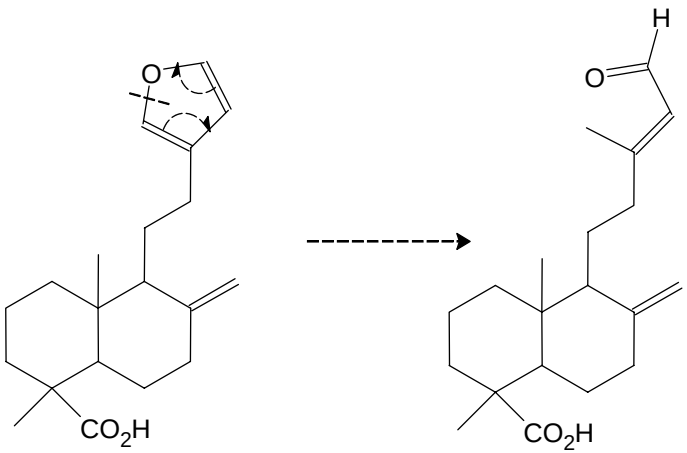


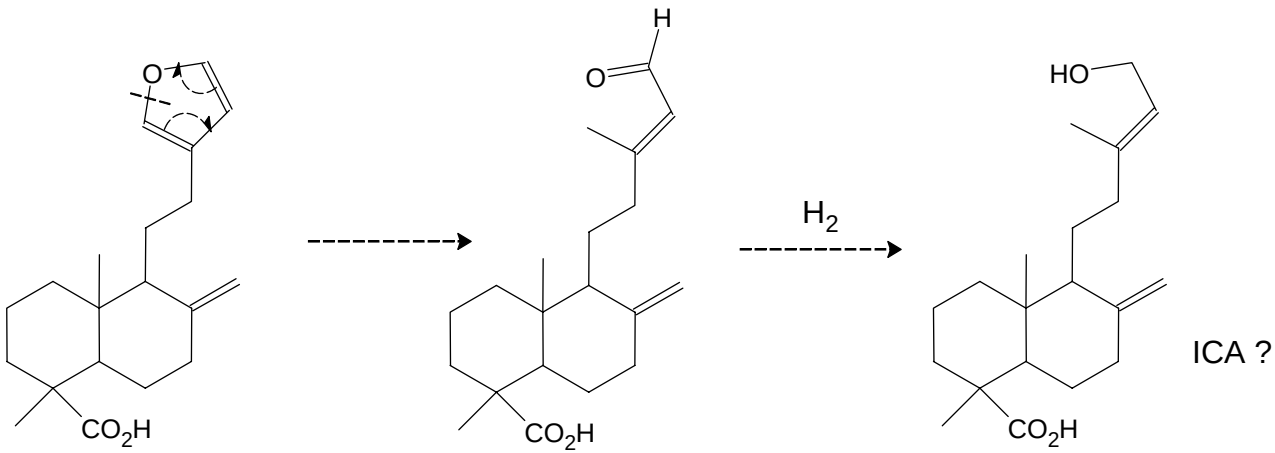


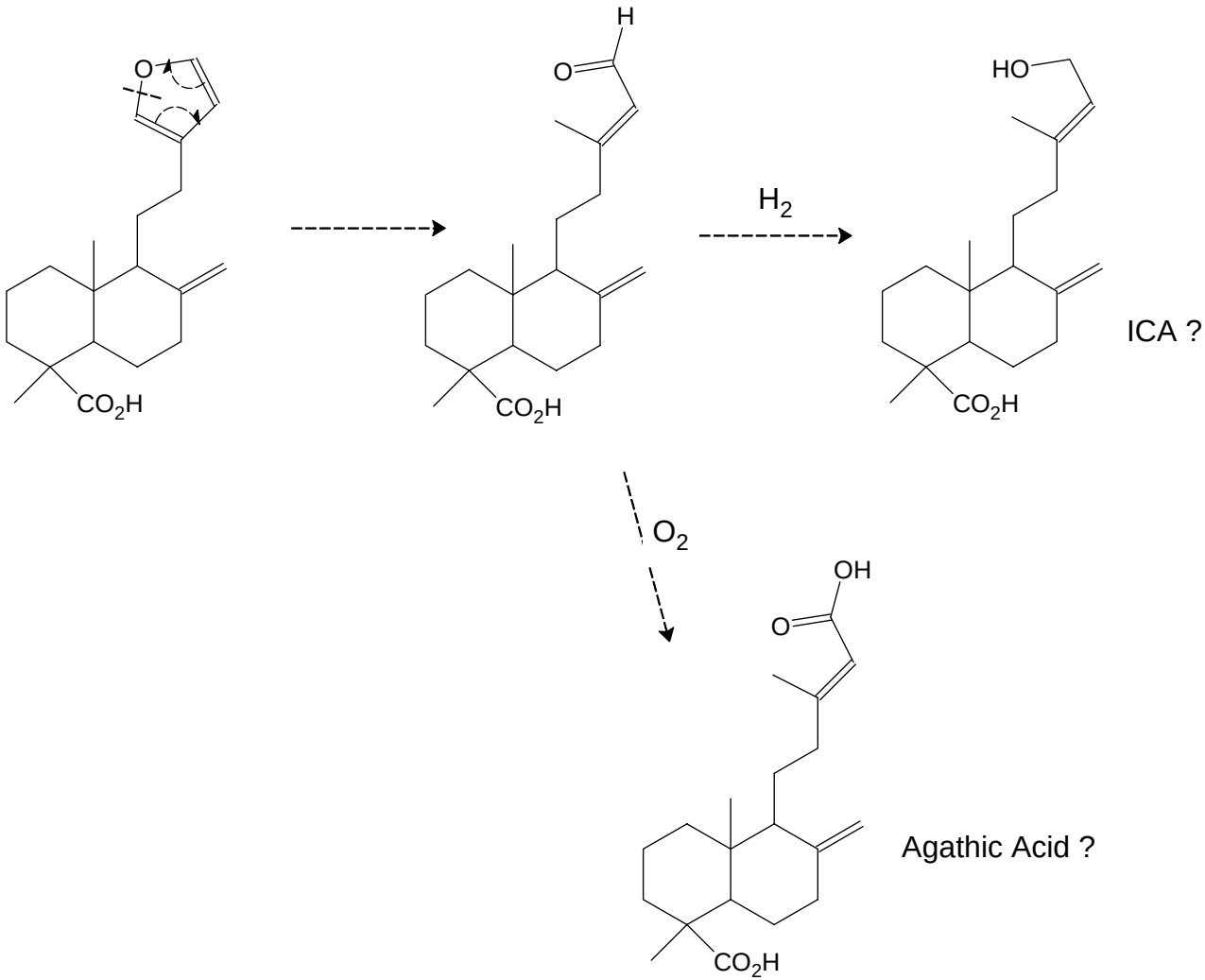
Isocupressic Acid



Polyalthic acid


O
CO2H
H
O
CO2H


O
H
O
HO
H_2
CO_2H
CO_2H
CO_2H
ICA ?


O
H
O
HO
H_2
CO_2H
CO_2H
CO_2H
ICA ?
O_2
OH
O
CO_2H
Agathic Acid ?