A Study on Antimalarial Activity of *Caesalpinia crista* and it's Chemical Constituents

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ငှက်ဖျားပိုးသေစေနိုင်သောအာနိသင်ရှိ
ကလိန်စေ့နှင့် ၎င်းတွင်
ပါဝင်သောဓာတုဒြပ်ပေါင်းများအားလေ့လာခြင်း

oef;aZmfvif* Shigetoshi Kadota**

*okwoet&m&SmewmOefcHI kwoeXme wuúodk&if;aq;wuúodkrfEÅav;

**Director, Institute of Natural Medicine, University of Toyama, Toyama, Japan.

edkjef;

- iSufzm;a&m*gonfrefmEdkifiHwGifomru wpfurÇmvHk;wGifvnf t"du

  udk;&m;aom usef;rma&;jyóemwpf&yfzpf

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- 3 oef;cefY ESifhatoqkH;&aoma&m*gzpf

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- iSufzsm;a&m*gukokq;0g;opfrsm; tvrvdktyfae
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  j'yfypfrsm; ukdbkwoef;ykdavhvmwifjyxm;
- uvdef;if (*Caesalpinia crista* Linn.) onfvbdkifaom obm0&ifaq;zuf&iftyif
- tjrpfyg wefnfukdtm;aq;? t qpfrpf&mif&m*gESifa;ema&m*grsm;wifo;H
- t ifa&;wiftaph uHdiSufzsm;a&m*gESifo;Haq; t jzpfo;Hu

- jrefm trnf - uvedfif
- ½kuA' trnf - *Caesalpinia crista* Linn.

  Caesalpinia bonducella L.
- t*vdf trnf - Gray nicker bean, Fever nut
- rdjif - Fabaceae/Caesalpiniaceae
- tonftyf - taph t&uftjrpf
- tyifH@mef - opfmcH, f

  yifnfudfzmrIm;

  q;Irm;pm&8

  &ufmpTkSfr;Eufyif

&nf;&Gf;csuf

- a,bk,s&nf;&Gf;csuf

  uvefpGmih iSifyg;ya&;u@o apEkdifaom tmeol iSifwkdkyfay;ifrsm; tm;&Sdaz azmfxkwfjcif;

- t"du &nf;&Gf;csuf

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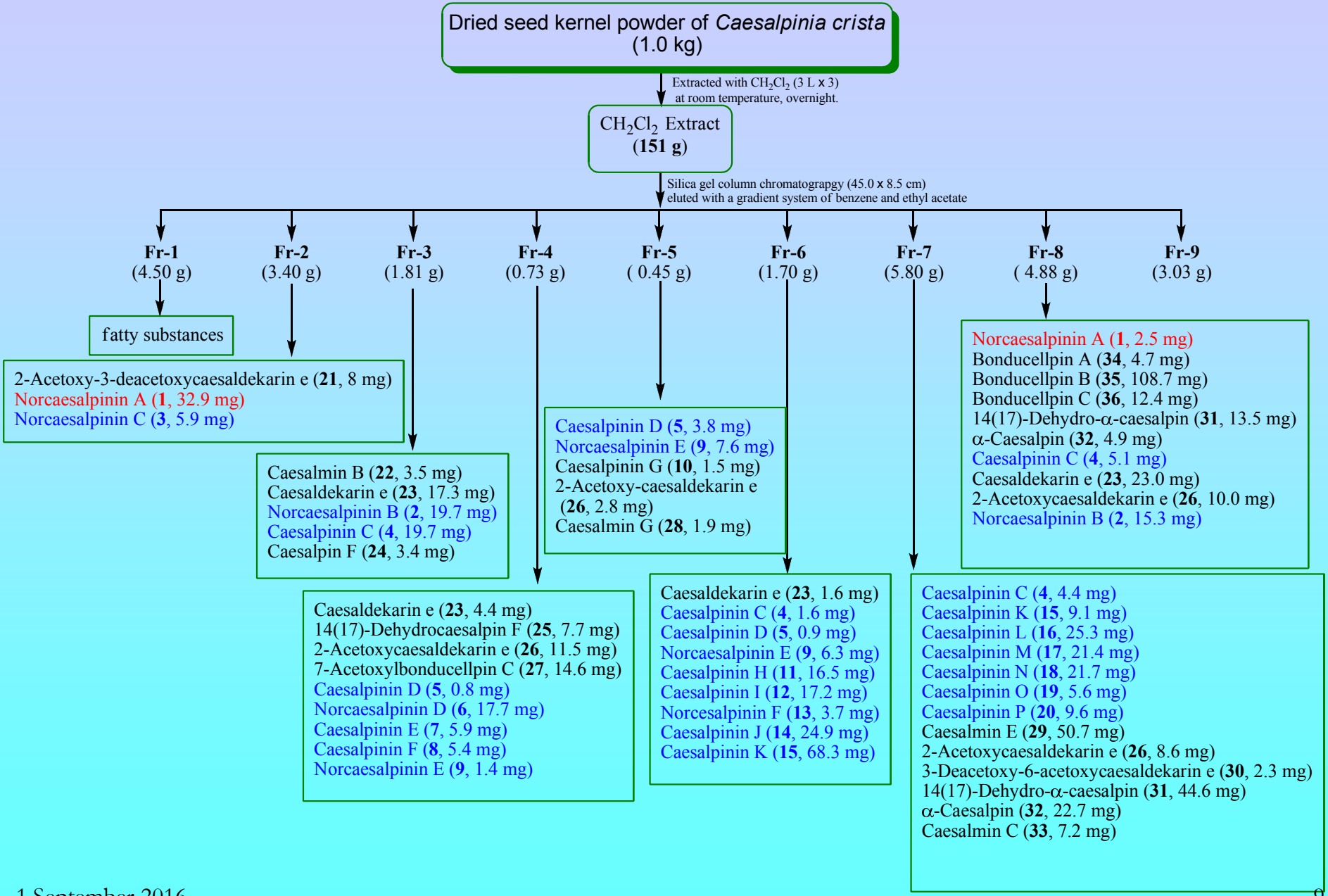
  2/ *uvefpGmhq;Wif;0ifaom "mwkdkyfay;ifrsm; tm;&Sdaz azmfxkwfjcif;*

  3/ *uvefpGmhq;Wif;0ifaom t"du "mwkdkyfay;if\ iSifyg;ya&;u@o apEkdifaom tmeol tm;&Sdaz azmfxkwfjcif;*

okawoejyKvkyfenf

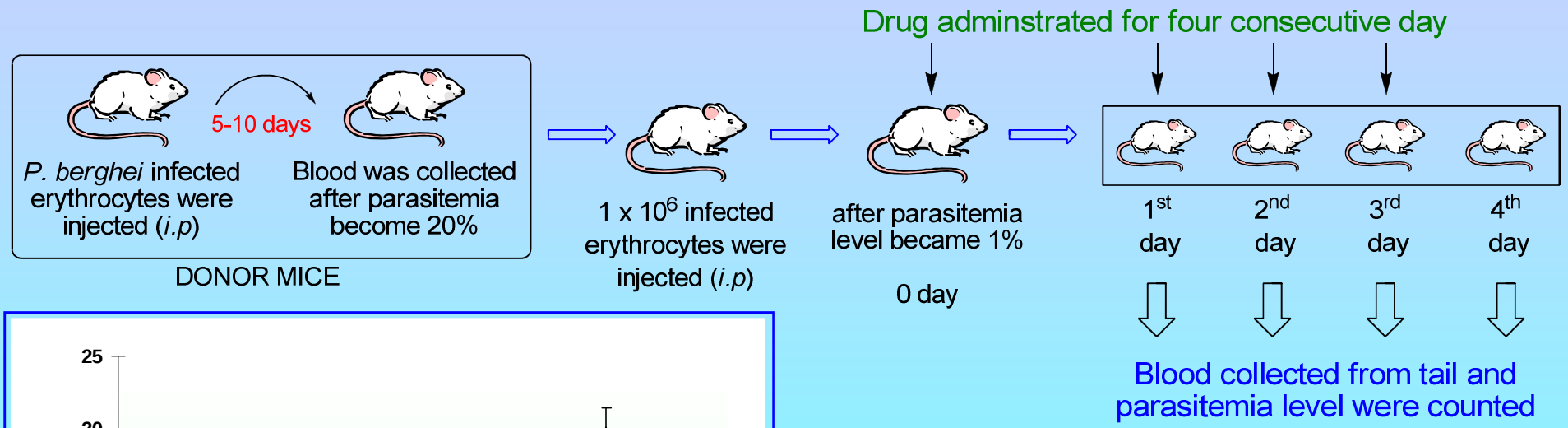
- ***1.aq;EpfSfxkwf,lcif;***(CH$_2$Cl$_2$ Extract)
- ***2.aq;EpfS\ iSufzsm;ydkyf tmedkifprfoyfcif;***(In-vivo, mice)
- ***3. aq;EpfSfwifyg0ifonfh'yfyifrsm;udkcGJxkwf,lcif;***(CC, PTLC, TLC)
- ***4. oefYpif'yfyifrsm;\ "mwkzGJUpnf;yHkudkqef;ppfjcif; (***Structure elucidation by NMR, MS, IR spectroscopic methods***)***
- ***5. t"dud'yfyif\ iSufzsm;ydkoapEkdifonfh tmedkifrIudkqef;ppfjcif;***
(Identification of active principle for antimalarial activity***)***

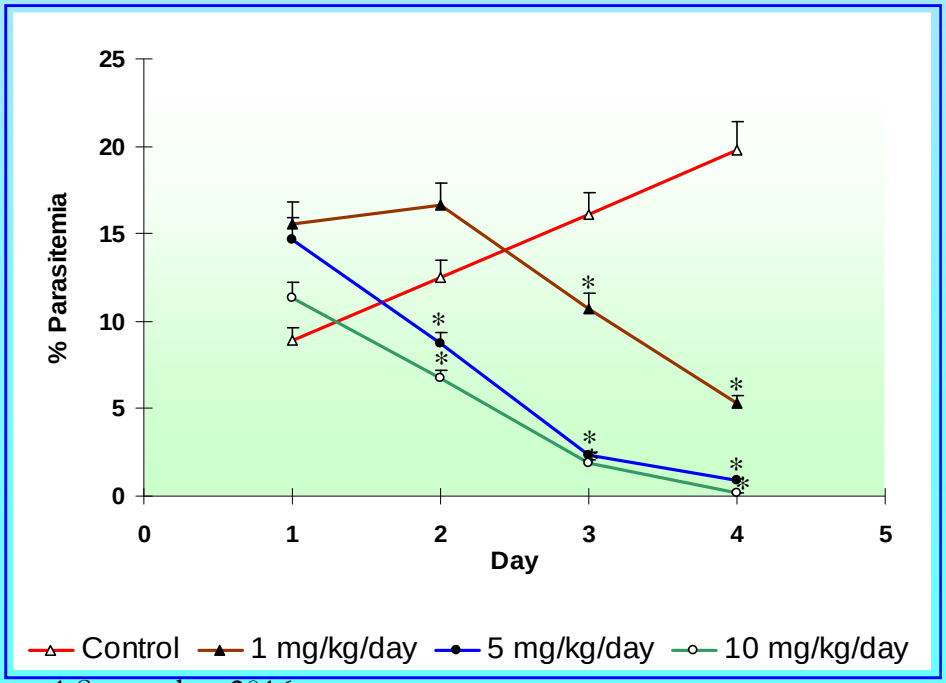
Extraction and isolation procedure of the seed kernel of *Caesalpinia crista*



An animal model used to study antimalarial activity of the CH_2Cl_2 extract against *Plasmodium berghei* infected mice *in vivo*

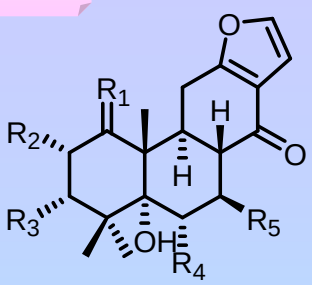
Animal: Balb/c male mice (7-8 weeks old) **Parasite**: *Plasmodium berghei* (strain Anka)



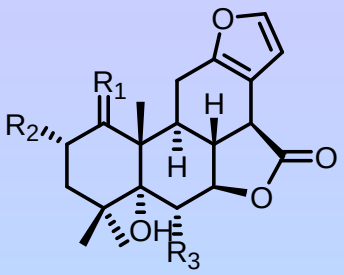


In each group three mice were taken and the results are shown with their mean value ± SD. * $p < 0.01$ significantly different from the control group by Student's *t*-test.

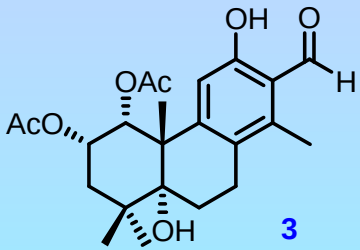
Structure of isolated compounds



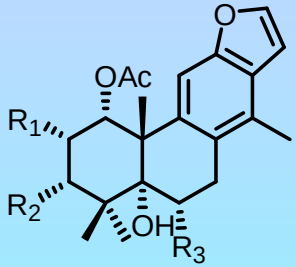
	R_1	R_2	R_3	R_4	R_5
1	α-OAc, β-H	OAc	H	H	H
2	α-OAc, β-H	H	OAc	H	H
6	α-OAc, β-H	OAc	OAc	H	H
9	α-OAc, β-H	H	H	H	OH
13	O	H	H	OAc	OH



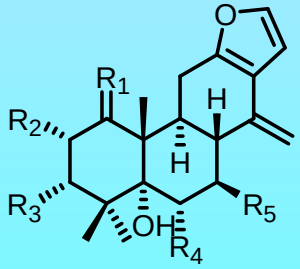
	R_1	R_2	R_3
5	α-OAc, β-H	H	OAc
10	α-OAc, β-H	OAc	H
11	α-OH, β-H	H	OAc
12	O	H	OAc
19	α-OAc, β-H	H	OH
22	α-OAc, β-H	H	H
28	α-OH, β-H	H	H



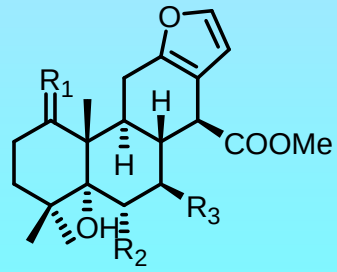
3



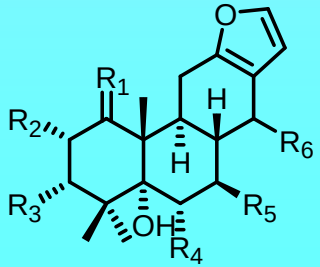
	R_1	R_2	R_3
21	OAc	H	H
23	H	OAc	H
26	OAc	OAc	H
30	H	H	OAc



	R_1	R_2	R_3	R_4	R_5
4	α-OAc, β-H	H	OAc	H	H
20	α-OAc, β-H	OAc	H	H	H
25	α-OAc, β-H	OAc	OAc	H	H
31	O	H	H	OAc	OAc
33	α-OAc, β-H	H	H	OAc	OAc



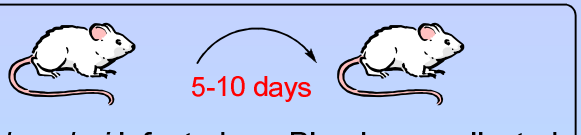
	R_1	R_2	R_3
7	α-OAc, β-H	OAc	H
8	O	OAc	H
14	O	OAc	OAc
17	α-OAc, β-H	OH	OAc
27	α-OAc, β-H	H	OAc
34	α-OAc, β-H	OAc	OH
35	O	OAc	OH
36	α-OAc, β-H	H	OH



	R_1	R_2	R_3	R_4	R_5	R_6
15	α-OAc, β-H	H	H	H	OH	α-CH_3, β-H
16	α-OAc, β-H	H	H	H	OAc	α-OH, β-CH_3
18	α-OAc, β-H	H	H	H	OH	α-H, β-CHO
24	α-OAc, β-H	OAc	OAc	H	H	α-CH_3, β-OH
29	α-OAc, β-H	H	H	OAc	OAc	α-CH_3, β-OH
32	O	H	H	OAc	OAc	α-OH, β-CH_3

An animal model used to study antimalarial activity of the Norcaesalpinin A (1)

Animal: ddY male mice (6-7 weeks old) **Parasite**: *Plasmodium berghei* (strain NK 65)

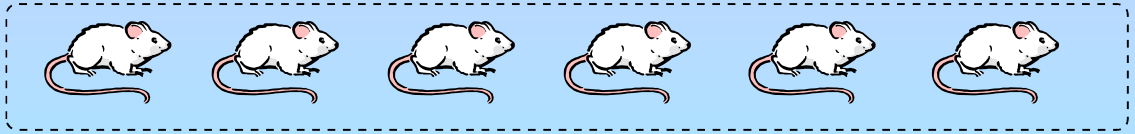


P. berghei infected erythrocytes were injected (*i.p*)

Blood was collected after parasitemia become 20%

DONOR MICE

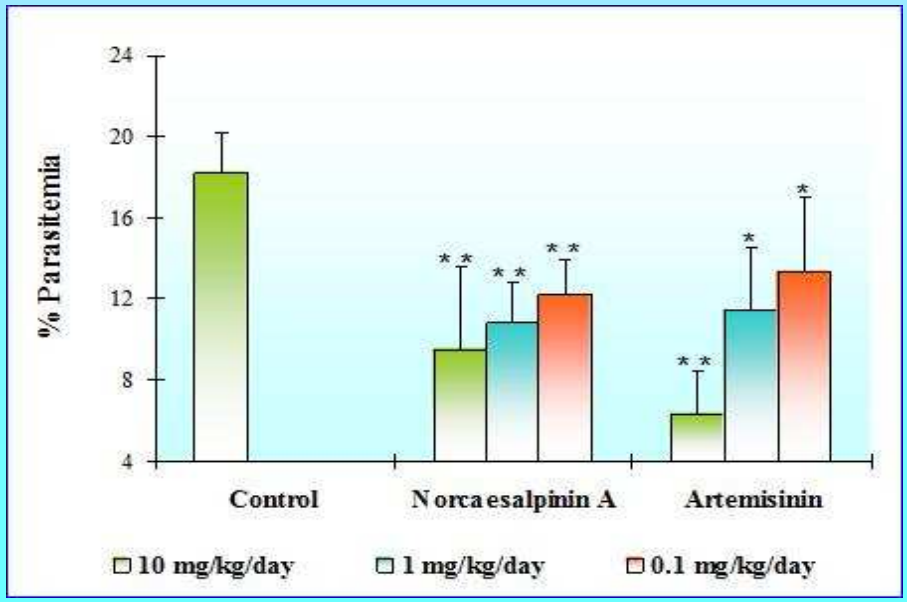
Drug adminstrated for seven consecutive day, the first administration was made 2 h after parasite inoculation

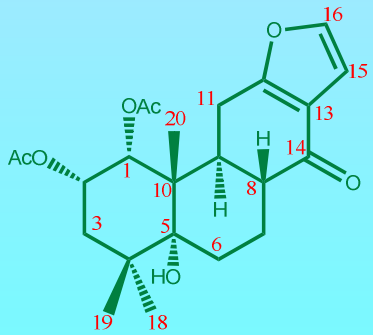


1 x 10^7 infected erythrocytes were injected (*i.v*)

6^{th} day

Blood collected from tail and parasitemia level were counted





In each group five mice were taken and the results are shown with their mean value ± SD.
** $p < 0.01$, * $p < 0.05$ significantly different from the control group by Student's *t*-test.

aq;,Gkdyfcsuf

- uvefpfh qH 'efuvsrD;aq;Ef (CH_2Cl_2 extract) wGifuyfuh&m isufq;ypfq;ubdkp maoapEdkif on tmeD ifawG&S
- 'efuvsrD;aq;Ef (CH_2Cl_2 extract) wif y0if on "mwkyfypf;ifrm;ul Isolation/ purification and Chromatography enf;pepfrm; toHk;í avhvmazmfxkwfcJh Cassane-type 'Gwmyi; (Diterpene) trsd;tpm; aq;yifxuf obm0'yfyif;ft opf trsd; (20) ty t0if "mwkyfyif; (36)rsdK cxkwf ,lEdkifcJh
- uvefpfh qH wGifyg0if on "mwkyfypf;ifrm;ESifh t"d "mwkyfyif; Norcaesalpinin-A \ "mwkyfpHkn;ylESif\ isufq;ypfo apEdkif on tmeD if (in-vivo antimalarial activity) udkrsm;wifyxrq;ESazmfxkwfawGU&Sh

- txuf tajccHq;okwoe (Experimental research) aw&Sufm;t& uvdkphqHnf iSfm;a&m*ay:wif tmediftumifawGaomfvnf aq;cdkyrmP rSufepmjzifh aq;vufwYkwoe (Clinical trial) qufvufyvjkdefvkHyfio;
- to;csokwoetwu tajccHkwoejzpfiom aq;yi\ j'yfyi;top &Sazaz:xlwf oHSHLSh twl tqHj'yfyif\ iSfm;yHkay: tmediftufa&mufq (in-vivo model) ukPf,Drfajrmufpmjzifvifyif...

au;Zl;wifvmT

- u vef;pD;bk;woeu;*yef;EdkifiHwGif aq;ukoaeaom todkif;tcsm. u,f;pkd;wdkif;a'oESifh 0ef;onf;a'oESifh nifrmMuD;wdkif;(Pm;jru:ES)0Ef;onf;rm tcsdef ;ydkif;rsm;wGif a&Sr;jznfh qμD q&mr u;rm; aiaAu;ESifh tppt&m&m axmufcHay;cJhaom *yef tjynfjynfqdkif&m yl;aygif;aqmif&Gufa&;at*sif pD (JICA) oifwef;rsm;o vrf;ñTefay;cJhaom *yef EdkifiH wdkif; mrkrf;rS wdkif; mr aq;EdkifiHq;0g;wuú odkifS q&mrm;tm; t xl;
au;Zl;wifaMumif ESifh , ckpm wrf;udkwifoGif; zwfonf;cifay;aom ñTefrm;a&;rS
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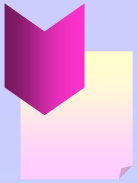
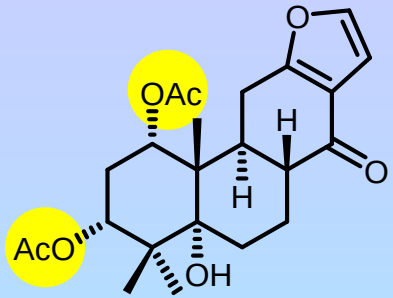
tyfygonf

- pmwrf;zwf;zpf;rmuf;a&; twufyjyfuhay;aom *&dwf0 xef;a&U; au oyfef rSwfEm;a&; aq;0g;xkwfvkyfa&; vkyfef;rd m;pk;m;uk nf t xl;au;Zl;wifygonf

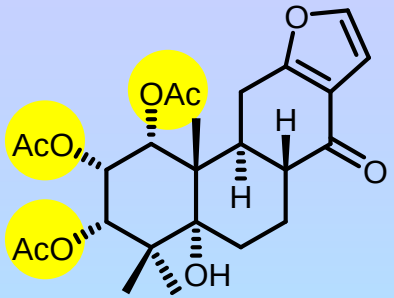
Thank you for your kind attention !



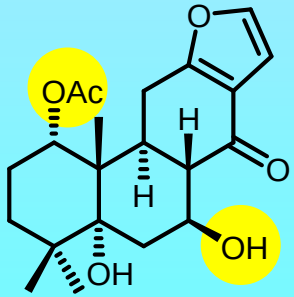
Norcaesalpinins B (2), D (6), E (9) and F (13)



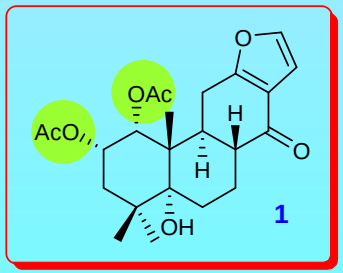
Norcaesalpinin B (**2**): colorless amorphous solid;
$[\alpha]_D^{25}$ +32.1 (c = 0.10, $CHCl_3$);
CD λ_{max} (2.87 x 10^{-4} M, EtOH) nm: 300 ([θ] +5337), 263 ([θ] -9757);
IR ($CHCl_3$) ν_{max} 3650, 1735, 1670 cm^{-1};
FABHRMS *m/z*: 419.2056 [calcd for $C_{23}H_{31}O_7$ $(M+H)^+$, 419.2070].

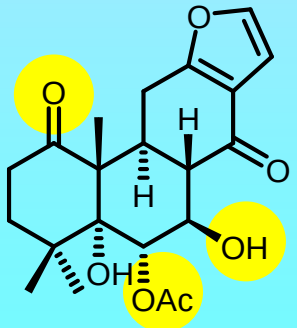


Norcaesalpinin D (**6**): colorless amorphous solid;
$[\alpha]_D^{25}$ +3.3 (c = 0.09, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1740, 1715 cm^{-1};
FABHRMS *m/z*: 477.2106
[calcd for $C_{25}H_{33}O_9$ $(M+H)^+$, 477.2125].



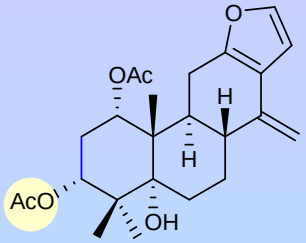
Norcaesalpinin E (**9**): colorless amorphous solid;
$[\alpha]_D^{25}$ +84.7 (c = 0.01, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1735, 1715 cm^{-1};
FABHRMS *m/z*: 377.1946
[calcd for $C_{21}H_{29}O_6$ $(M+H)^+$, 377.1964].



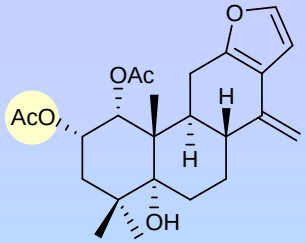


Norcaesalpinin F (**13**): colorless amorphous solid;
$[\alpha]_D^{22}$ +80.37 (c = 0.091, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3600, 1740, 1710 cm^{-1};
FABHRMS *m/z*: 391.1749
[calcd for $C_{21}H_{27}O_7$ $(M+H)^+$, 391.1757].

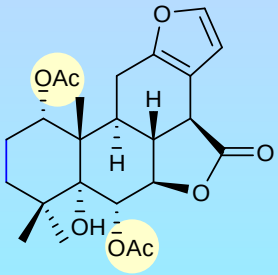
Structure of caesalpinins



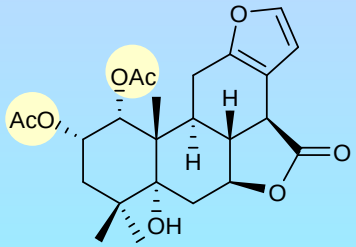
Caesalpinin C (**4**): colorless amorphous solid;
$[\alpha]_D^{25}$ +30.2 (c = 0.11, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1735 cm^{-1};
FABHRMS *m/z*: 417.2314 [calcd for $C_{24}H_{33}O_6$ $(M+H)^+$, 417.2277].



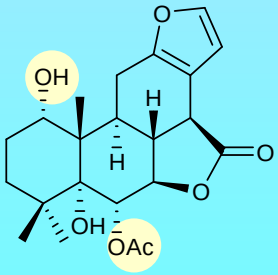
Caesalpinin P (**20**): colorless amorphous solid;
$[\alpha]_D^{22}$ +11.64 (c = 0.074, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1730 cm^{-1};
FABHRMS *m/z*: 417.2294 [calcd for $C_{24}H_{33}O_6$ $(M+H)^+$, 417.2277].



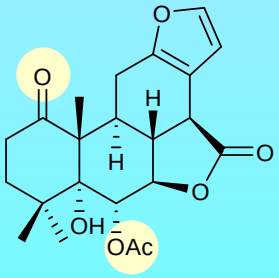
Caesalpinin D (**5**): colorless amorphous solid;
$[\alpha]_D^{25}$ +63.2 (c = 0.057, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1750, 1735 cm^{-1};
FABHRMS *m/z:* 447.2025 [calcd for $C_{24}H_{31}O_8$ $(M+H)^+$, 447.2031].



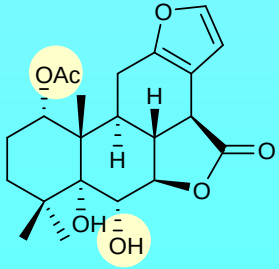
Caesalpini G (**10**): colorless amorphous solid;
$[\alpha]_D^{25}$ +58.2 (c = 0.063, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1750, 1735 cm^{-1};
FABHRMS *m/z*: 447.2009 [calcd for $C_{24}H_{31}O_8$ $(M+H)^+$, 447.2019].



Caesalpinin H (**11**): colorless amorphous solid;
$[\alpha]_D^{25}$ +67.5 (c = 0.057, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1750, 1735 cm^{-1};
FABHRMS *m/z*: 405.1915 [calcd for $C_{22}H_{29}O_7$ $(M+H)^+$, 405.1913].

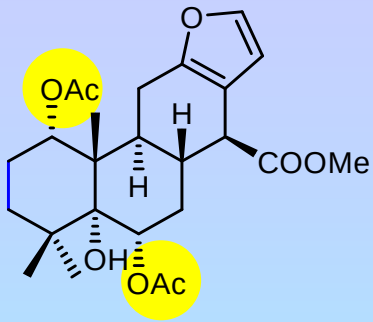


Caesalpinin I (**12**): colorless amorphous solid;
$[\alpha]_D^{22}$ +59.73 (c = 0.053, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3600, 1755, 1710 cm^{-1};
FABHRMS *m/z*: 403.1792 [calcd for $C_{22}H_{27}O_7$ $(M+H)^+$, 403.1757].

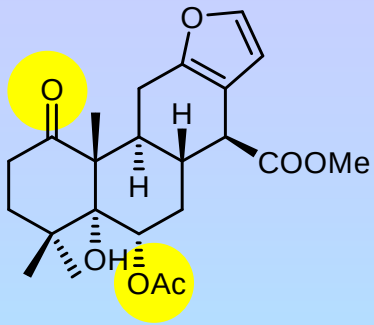


Caesalpinin O (**19**): colorless amorphous solid;
$[\alpha]_D^{22}$ +56.76 (c = 0.078, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1750, 1735 cm^{-1};
FABHRMS *m/z*: 405.1929 [calcd for $C_{22}H_{29}O_7$ $(M+H)^+$, 405.1913].

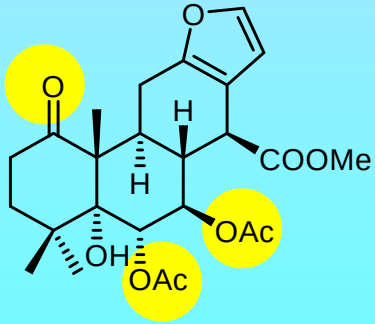
Caesalpinin E (7), F (8), J (14) and M (17)



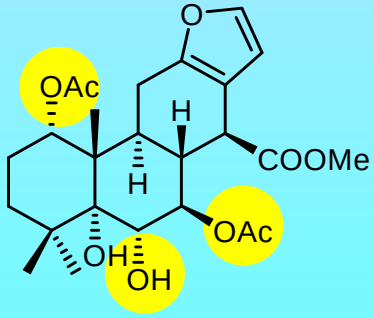
Caesalpinin E (**7**): colorless amorphous solid;
$[\alpha]_D^{25}$ +125.98 (*c* = 0.02, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1735 cm^{-1};
FABHRMS *m/z*: 463.2317
[calcd for $C_{25}H_{35}O_8$ $(M+H)^+$, 463.2332].



Caesalpinin F (**8**): colorless amorphous solid;
$[\alpha]_D^{25}$ +47.0 (*c* = 0.081, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1730, 1710 cm^{-1};
FABHRMS *m/z*: 419.2051
[calcd for $C_{23}H_{31}O_7$ $(M+H)^+$, 419.2027].

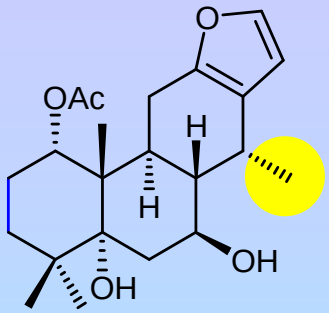


Caesalpinin J (**14**): colorless amorphous solid;
$[\alpha]_D^{22}$ +42.03 (*c* = 0.088, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1735, 1715 cm^{-1};
FABHRMS *m/z*: 477.2130
[calcd for $C_{25}H_{33}O_9$ $(M+H)^+$, 477.2124].

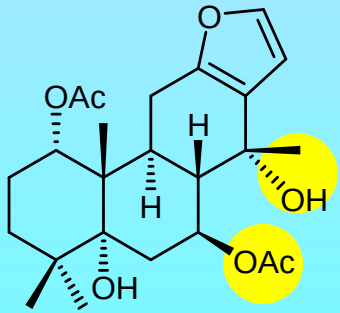


Caesalpinin M (**17**): colorless amorphous solid;
$[\alpha]_D^{22}$ +47.13 (*c* = 0.074, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1735 cm^{-1};
FABHRMS *m/z*: 479.2272
[calcd for $C_{25}H_{35}O_9$ $(M+H)^+$, 479.2281].

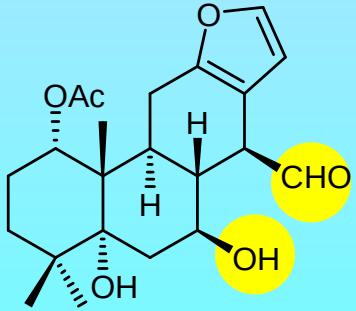
Caesalpinin K (15), L (16) and N (18)



Caesalpinin K (**15**): colorless amorphous solid;
$[\alpha]_D^{22}$ +51.54 (c = 0.151, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1730 cm^{-1};
FABHRMS *m/z*: 377.2314
[calcd for $C_{22}H_{33}O_5$ $(M+H)^+$, 377.2328].



Caesalpinin L (**16**): colorless amorphous solid; $[\alpha]_D^{22}$ +37.83 (c = 0.171, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1735 cm^{-1};
FABHRMS *m/z*: 435.2336 [calcd for $C_{24}H_{35}O_7$ $(M+H)^+$, 435.2383].



Caesalpinin N (**18**): colorless amorphous solid; $[\alpha]_D^{22}$ +28.8 (c = 0.195, $CHCl_3$);
IR ($CHCl_3$) ν_{max} 3575, 1735, 1715 cm^{-1};
FABHRMS *m/z*: 391.2096 [calcd for $C_{22}H_{31}O_6$ $(M+H)^+$, 391.2121].

Conclusion

- The CH_2Cl_2 extract showed significant inhibition of parasitemia level in the mice infected with *plasmodium berghei* (strain Anka).

- The chemical examination of CH_2Cl_2 extract led to the isolation of thirty-six cassane-type diterpenes, among which six compounds (**1**, **2**, **3**, **6**, **9** and **13**) represent unprecedented carbon framework. Norcaesalpinin A (**1**), B (**2**), D (**6**), E (**9**) and F (**13**) had 17-norcassane skeleton, while norcaesalpinin C (**3**) had 16-norcassane skeleton and rest fourteen were cassane-type new compounds.

- The antimalarial activity of norcaesalpinin A (1) was examined in mice infected with *P. berghei* (strain NK 65) in 6-day suppressive test. At a dose of 10, 1 and 0.1 mg/kg/day (*p.o.*), norcaesalpinin A (1) showed significant dose dependent inhibition of parasitemia level. These results suggested that the antimalarial activity of the CH_2Cl_2 extract of *C. crista* may possibly be due to cassane-type diterpenes.

Introduction

- Malaria is a parasitic disease affecting 200-300 million people in the tropical and subtropical regions of the world which claims the lives of approximately three million each year.
- *Caesalpinia crista* Linn. (Fabaceae), commonly known as Kalain(***uvefif***, is widely distributed throughout the tropical and subtropical regions. People in local communities use its seed kernel as anthelmintic and antimaliarial drugs.
- Present study aimed to find an active constituents from *Caesalpinia crista* and it's antimalarial activity.

- In Myanmar, the National Health Plan has been implemented by the Ministry of Health under the guidance of the Head of State. As such, the NHP has been aimed to solve the problem of six diseases identified as top priority in Myanmar in accordance with their prevalence and their burden inflicted upon the health sector. One of the objectives is to effectively control and treat the diseases utilizing the locally available resources, including traditional drugs and indigenous practices. The six priority diseases include:

- (1) Malaria
- (2)Tuberculosis
- (3) Diabetes
- (4) Hypertension
- (5) Diarrhoea and
- (6) Dysentery

- Globally, during the past decade, it has been increasingly recognized that modern allopathic medicine itself is insufficient to solve many aspects of the current health problems and the WHO has envisaged the upgrading and involvement of traditional medicine as an alternative medicine to serve as a complementary arm within the current health care sector. The main aim and duty of any doctor is to alleviate the ailments of his patients without inflicting untoward effects. The same goes with traditional medicine physicians, and it thus, becomes a necessity to prevent the loss of valuable traditional remedies with time due to underuse, misuse and abuse following the lack of research, documentation and quality control.

- Myanmar traditional medicine has stood a long history of self-sufficiency even before the use of western medicine. Even under the colonial rule, prominent traditional practitioners like Saya San, have kept the traditional practices alive and active.
- When the State Peace and Development Council took over, the Head of State has taken a very keen interest in the upgrading and supporting traditional medicine and country-wide traditional medicine conferences and exhibitions were held for public awareness towards Myanmar traditional medicine and its healing ability.

- The Department of Medical Research (Lower Myanmar, Middle Myanmar and Upper Myanmar) and Department of Traditional Medicine have undertaken the responsibility of conducting research and utilization of locally available traditional remedies in the treatment of the 6 priority diseases of Myanmar for many years.

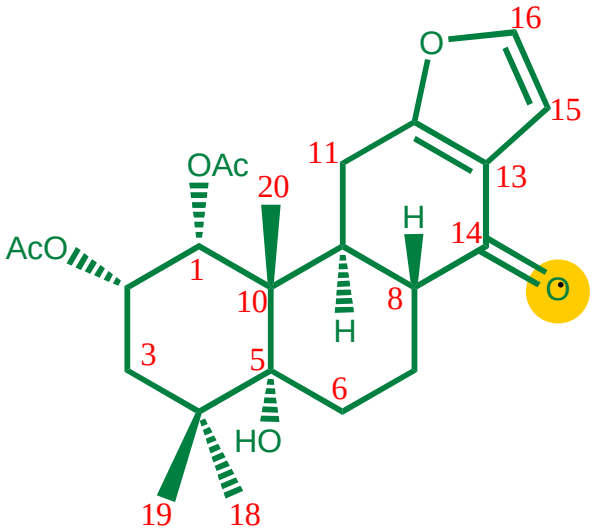
Malaria

- With the global upsurge of malaria within the last decade, malaria is considered the number one priority disease in Myanmar. In addition, the increasing problem of multi-drug resistance has added a greater burden, not only on the individual patients, but also for malaria control in general, especially when cost and availability is concerned. With the aim to increase the utilization of locally available resources and attainment of self-sufficiency, the State Peace and Development Council, under the guidance of the Head of State, has formed a committee concerning the development of drugs against malaria using locally available resources.

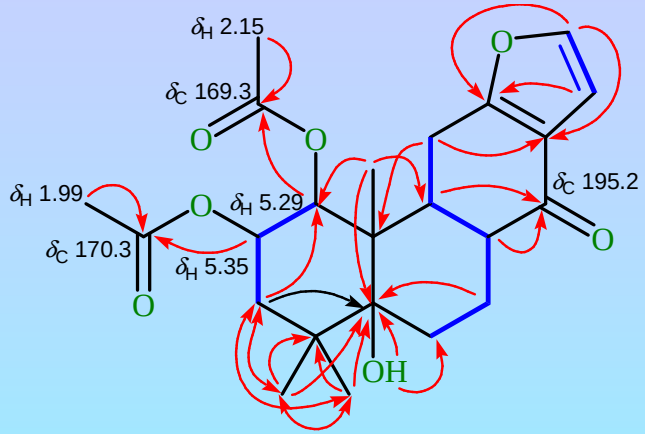
- The role of the Ministry of Health, as a member in this committee, is concerned with the aspect of disease control, treatment of malaria, drug production/procurement and finally, to conduct research against malaria. The outputs of such research were expected to contribute to the Ministry of Industry (1) in the production of effective antimalarial drugs in future. Since 1984, the Department of Medical Research (Lower Myanmar) has been conducting research on various plants and traditional medicine, reputed to have antimalarial action, including locally available *Artemisia annua* (***eåheRWwå***) and the production of quinine from locally available cinchona trees.

Norcaesalpinin A (1)

Colorless amorphous solid; $[\alpha]_D^{25}$ -20.1 (c = 0.15, $CHCl_3$);
CD λ_{max} (2.51×10^{-4} M, EtOH) nm: 302 ([θ] +6993), 263 ([θ] -8597);
IR ($CHCl_3$) ν_{max} 3625, 1735, 1670 cm^{-1};
HRFABMS m/z: 419.2073 [calcd for $C_{23}H_{31}O_7$ $(M+H)^+$, 419.2070].

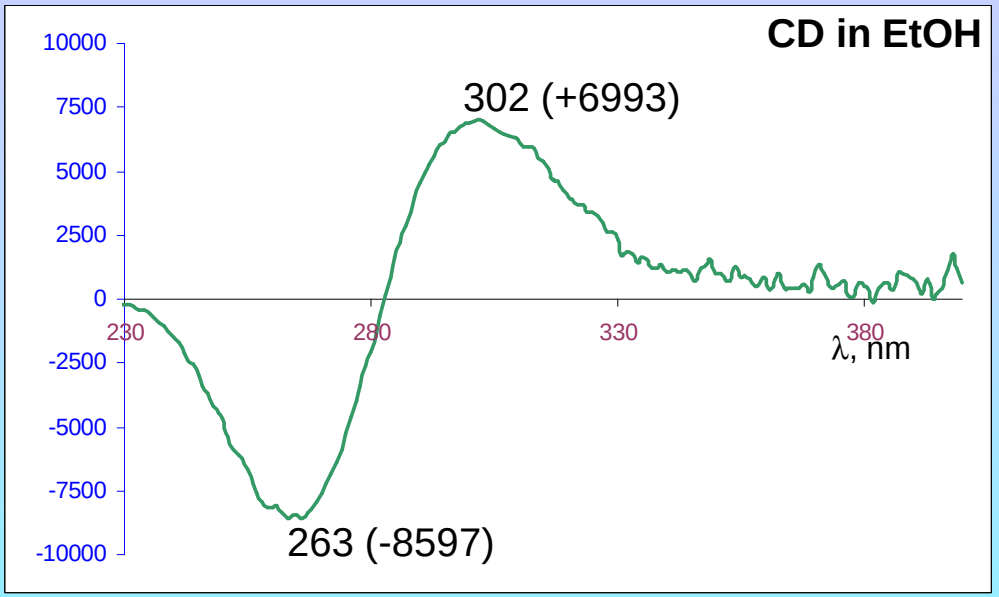


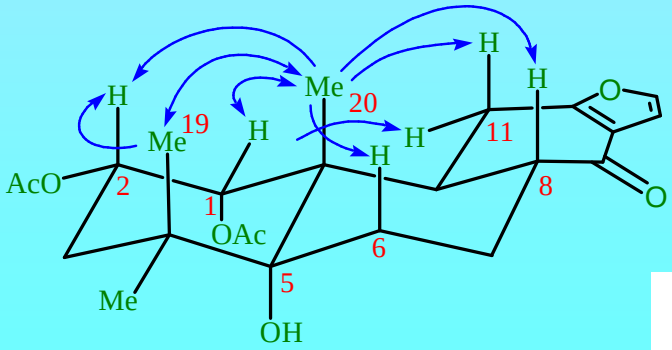
HMBC, ^{1}H-^{1}H COSY and NOE correlations for Norcaesalpinin A (1)



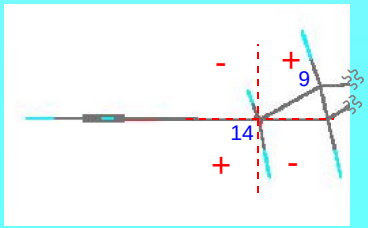
: HMBC correlations

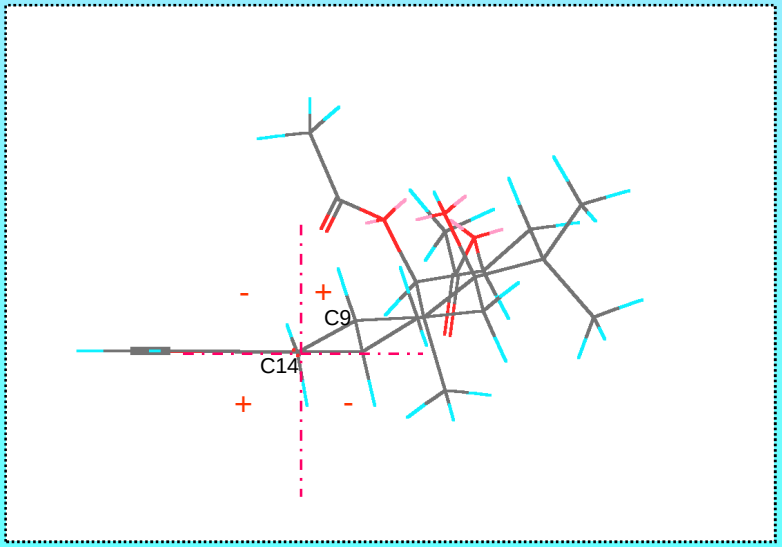
: ^{1}H-^{1}H COSY correlations





Significant NOE observed
in difference NOE experiment



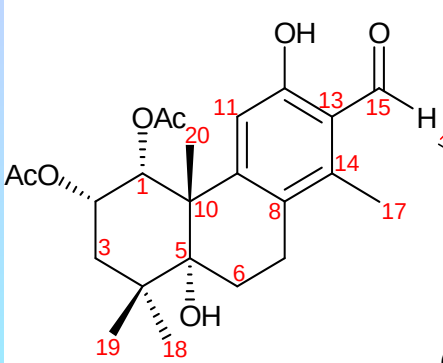


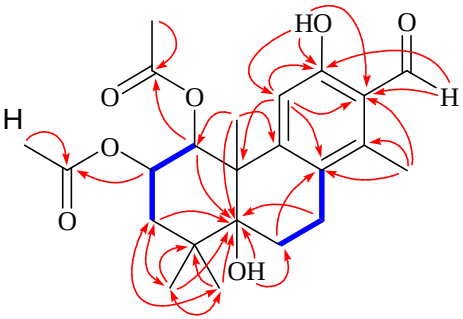
^{1}H-NMR, HMBC, ^{1}H-^{1}H COSY and NOE correlations for Norcaesalpinin C (3)

colorless amorphous solid; $[\alpha]_D^{25}$ –55.3° (c = 0.10, $CHCl_3$);

IR ($CHCl_3$) ν_{max} 3575, 1785, 1640 cm^{-1};

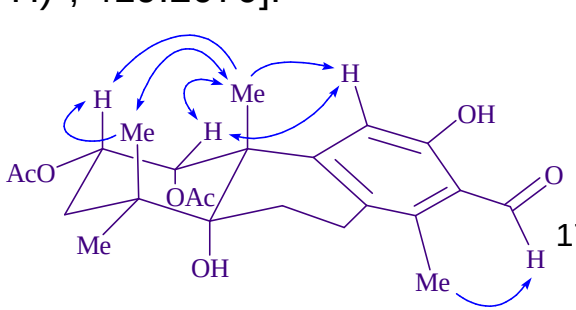
FABHRMS m/z: 419.2084 [calcd for $C_{23}H_{31}O_7$ $(M+H)^+$, 419.20ｲUJ.





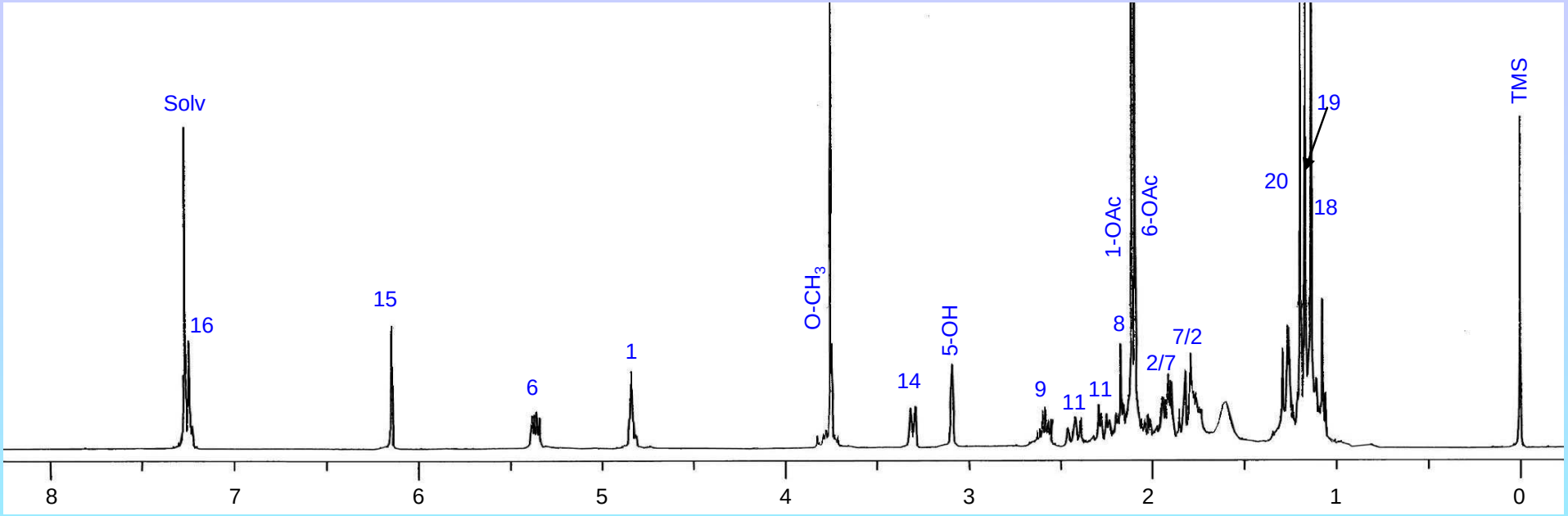
(red arrow) = HMBC correlations

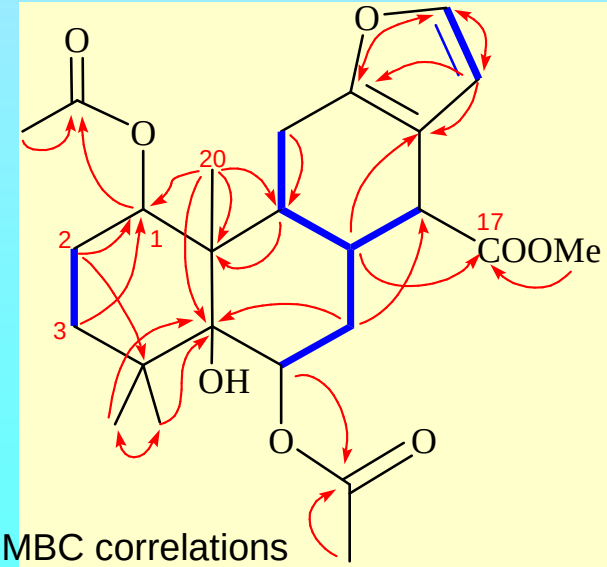
(Blue bold line) = ^{1}H-^{1}H COSY correlation

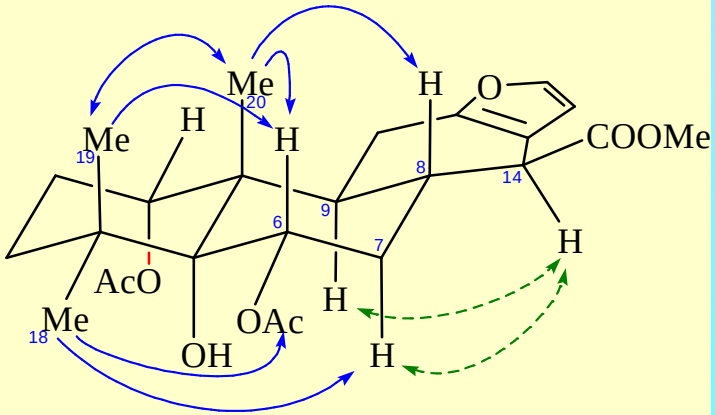


(blue arrow) = NOE

^{1}H NMR, COSY HMBC, NOE and ROESY of Caesalpinin E (7)







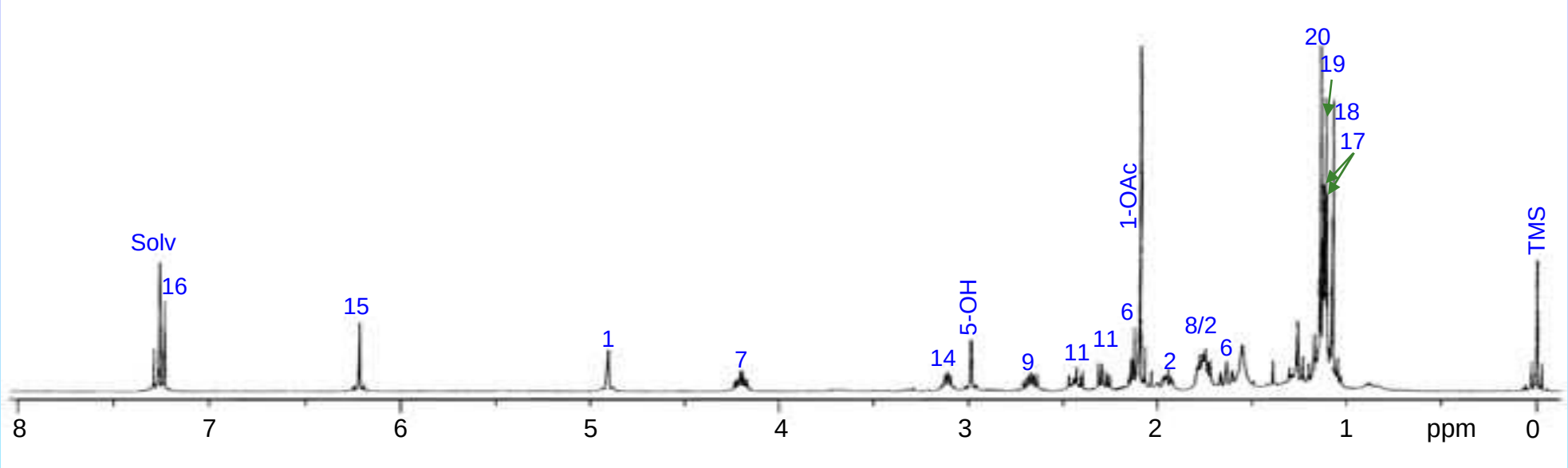
: NOE

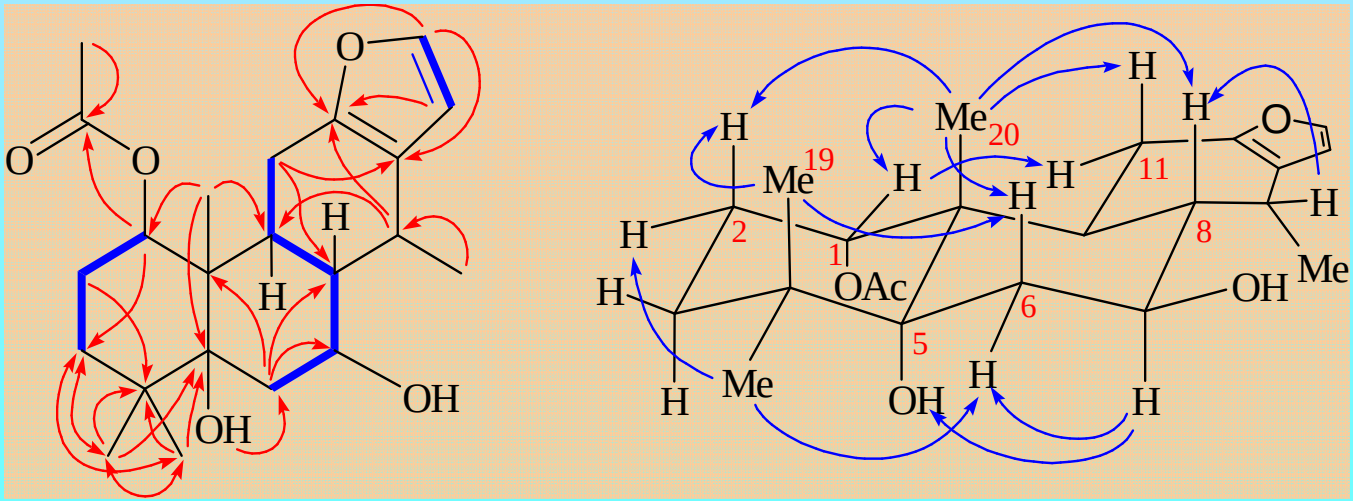
: ROESY

(red arrow) = HMBC correlations

(Blue bold line) = ^{1}H-^{1}H COSY correlation

^{1}H NMR, COSY HMBC, NOE and ROESY of Caesalpinin K (15)





(red arrow) = HMBC correlations
(Blue bold line) = ^{1}H-^{1}H COSY correlation

(blue arrow) = NOE