KIMIA BAHAN ALAM
(CHEMISTRY OF NATURAL PRODUCT)

TRITERPENOID DAN STEROID
BIOSINTESIS, ISOLASI, DAN KARAKTERISASI

By
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Kontrak Kuliah

Dosen: Masruri, S.Si., M.Si., PhD
Penilaian: UTS (40%), Tugas-1 (20%), Tugas-2 (20%), Tugas-3 (20%)
Quesioner mahasiswa: Diberikan saat UTS

Materi: (sesui dengan Buku Pedoman Pendidikan)
Pendahuluan --- sesi 1
Triterpenoid (biosintesis, isolasi dan karakterisasi) -- sesi 2-3
Poliketida (biosintesis, isolasi dan karakterisasi) – sesi 4-5
Alkaloid (biosintesis, isolasi dan karakterisasi) – sesi 6-7
UTS

Terpenoid
Fenolik
Flavonoid
Fenil propanoid
UAS
Examples of Natural Products as Leads & Drugs

Cardiac glycosides, morphine, quinine, salicylic acid, taxol, camptothecin, penicillin, cyclosporin A, warfarin, artemisine….

OHO
R = H: Morphine
R = Me: Codeine
(pain killer)

HO
O
OH
H
H
H
H
H

17-ethynylestradiol norethindrone
(the "pill" contraceptive)

Clarithromycin
(antibacterial)

HO
O
O
OMe
HO
O
O
OMe
OH
O
N
S
O
NH2
CO2H
H
H
Ampicillin
(antibiotic)

Clavulanic acid
(β-lactamase inhibitor)

Augmentin
(antibiotic)

Cyclosporine A

Triterpenoids as multifunctional agents for the prevention and treatment of cancer

Triterpenoids as multifunctional agents for the prevention and treatment of cancer


Simple building blocks serve as the basis for each major pathway of secondary metabolism:

(1) **Shikimate** → Aromatics (ring - C<sub>3</sub> chain)

(2) **Amino acids** → Alkaloids, Penicillins (N-containing)

(3) **Mevalonate** → Terpenes, Steroids

(4) **Acetate** → Polyketides (aromatics, macrocycles)

AAML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CDDO, 2-cyano-3,12-dioxoolean-1,8(13)-diene-20-acid; CDDO-ile, CDDO-imidazole; CDDO-Me, CDDO-methyl ether; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; N/A, not applicable.

**CLASSIFICATION OF TERPENES**

<table>
<thead>
<tr>
<th>TYPE OF TERPENE</th>
<th>NUMBER OF CARBON ATOMS</th>
<th>ISOPRENE UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemiterpene</td>
<td>C₅</td>
<td>one</td>
</tr>
<tr>
<td>monoterpene</td>
<td>C₁₀</td>
<td>two</td>
</tr>
<tr>
<td>sesquiterpene</td>
<td>C₁₅</td>
<td>three</td>
</tr>
<tr>
<td>diterpene</td>
<td>C₂₀</td>
<td>four</td>
</tr>
<tr>
<td>sesterterpene</td>
<td>C₂₅</td>
<td>five</td>
</tr>
<tr>
<td>triterpene</td>
<td>C₃₀</td>
<td>six</td>
</tr>
<tr>
<td>tetraterpene</td>
<td>C₄₀</td>
<td>eight</td>
</tr>
</tbody>
</table>

**NOTE:**
- hemi = half
- sesqui = one and a half
- di = two
- tri = three
- tetra = four
TRITERPENOID

- Termasuk dalam metabolit sekunder terpenoid.
- Tersusun atas 6 unit isoprenoid (C5), atau mengandung 30 karbon, (C30).
- Biosintesis triterpenoid tidak melalui penggabungan satu per satu unit isoprena (IPP). Tetapi penggabungan antara dua unit C15. Yaitu melalui farnesilpiroposfat (FPP)

**How terpenoid is formed**

- IPP
- DMAPP
- C5: isopenteny-PP
- 3,3-dimethylallyl-PP
- C10: geranyl-PP
- C15: farnesyl-PP
- C20: geranyl-geranyl-PP

Each new unit is joined head-to-tail.
BIOSINTESIS

TRITERPENES

amberin
ambergis

Siklisasi squalena

Dewick, 2002
Stereochemistry control biosynthesis products

Dewick, 2002
Stereochemistry control biosynthesis products

Euphol from *Euphorbia* species (Euphorbiaceae)

Dammarenediols, was found in Dammar resin from *Balanocarpus heimii* (Dipterocarpaceae) and ginseng (*Panax ginseng*; Araliaceae)

Lupeol, found in lupin (*Lupinus luteus*; Leguminosae/Fabaceae)

Taraxasterol found in dandelion (*Taraxacum officinale*; Compositae/Asteraceae)

Dewick, 2002
**Triterpenoid saponin**

The pentacyclic triterpenoid skeletons such as lupeol, α-amyrin, and β-amyrin are triterpenoid saponin structures. Saponins are glycosides which, even at low concentrations, produce a frothing in aqueous solution. They have surfactant and soaplike properties. Latin *sapo*, soap, and plant materials containing saponins.

**Modified triterpeneoid**

The triterpenoid skeletons may be subjected to a variety of structural modifications. They loss of several skeletal carbon atoms.

Limonoids was found in families Rutaceae, Meliaceae, and Simaroubaceae. **Azadirachtin**, from Neem tree (*Azadirachta indica*; Meliaceae) for use as an agricultural pesticide to prevent insect damage to crops.

Quassinoids are produced in Simaroubaceae, *Quassia*. **Quassin** from *O. amara* (quassia wood). They have cytotoxic, antimalarial, and amoebicidal properties.
STERIOIDS ARE TRITERPENES (C30)

All steroids are triterpenes but their skeletons have been rearranged so that they can not be analyzed into isoprene units.

Chemical Constituents from the Leaves of *Annona reticulata* and Their Inhibitory Effects on NO Production

Tran Dinh Thang, Ping-Chung Kuo, Guan-Jhong Huang, Nguyen Huy Hung, Bow-Shin Huang, Mei-Lin Yang, Ngo Xuan Luong and Tian-Shung Wu, *Molecules*, 2013, 18, 4477-4486; doi:10.3390/molecules18044477
Chemical Constituents from the Leaves of *Annona reticulata* and Their Inhibitory Effects on NO Production

Figure 1. Structure and significant HMBC (→) correlations of compound 1.

![Chemical Structure](image)

Table 1. Inhibitory effects of purified samples from the leaves of *A. reticulata* on LPS-induced iNOS-dependent NO production in RAW 264.7 cells.

<table>
<thead>
<tr>
<th></th>
<th>Dose (µM)</th>
<th>Cell viability (% of control)</th>
<th>NO level</th>
<th>NO inhibition (% of control)</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>100.0 ± 4.9</td>
<td>-0.5 ± 0.1</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>+</td>
<td>98.7 ± 8.0</td>
<td>45.4 ± 2.7</td>
<td>(-)</td>
</tr>
<tr>
<td>1</td>
<td>12.5</td>
<td>88.0 ± 1.5</td>
<td>36.6 ± 1.9</td>
<td>19.3 ± 4.2</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>81.2 ± 1.8</td>
<td>33.5 ± 0.9</td>
<td>26.2 ± 2.0</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>73.0 ± 3.6</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>66.9 ± 5.5 ++</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>98.0 ± 2.2</td>
<td>34.3 ± 4.7</td>
<td>24.5 ± 10.2</td>
<td>50.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>91.0 ± 3.6</td>
<td>35.1 ± 1.2</td>
<td>22.7 ± 2.6</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>82.9 ± 1.2</td>
<td>22.7 ± 0.5**</td>
<td>50.0 ± 1.0</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>65.7 ± 6.6 **</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>93.5 ± 1.8</td>
<td>38.5 ± 2.3</td>
<td>15.2 ± 5.0</td>
<td>99.8 ± 0.4</td>
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<tr>
<td></td>
<td>25</td>
<td>92.6 ± 3.1</td>
<td>39.5 ± 0.6</td>
<td>13.1 ± 1.2</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>88.7 ± 1.8</td>
<td>35.6 ± 0.9**</td>
<td>21.6 ± 2.0</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>81.4 ± 2.3</td>
<td>22.6 ± 0.4**</td>
<td>50.1 ± 0.9</td>
<td>(-)</td>
</tr>
<tr>
<td>4</td>
<td>12.5</td>
<td>82.8 ± 2.7</td>
<td>38.4 ± 0.7</td>
<td>13.5 ± 1.5</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>80.7 ± 3.8</td>
<td>38.6 ± 3.3</td>
<td>15.1 ± 7.3</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>65.3 ± 2.3 **</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>55.8 ± 2.7 **</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>
Chemical Constituents from the Leaves of *Annona reticulata* and Their Inhibitory Effects on NO Production

**Table 1. Inhibitory effects of purified samples from the leaves of *A. reticulata* on LPS-induced NO production in RAW 264.7 cells.**

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<tr>
<td>LPS</td>
<td>98.7 ± 0.0</td>
<td>45.4 ± 2.7**</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>4</td>
<td>83.0 ± 2.7</td>
<td>38.4 ± 0.7</td>
<td>15.5 ± 1.5</td>
<td>(-)</td>
</tr>
<tr>
<td>25</td>
<td>80 ± 7.8</td>
<td>38.6 ± 3.3</td>
<td>15.1 ± 7.3</td>
<td>(-)</td>
</tr>
<tr>
<td>50</td>
<td>85.3 ± 2.3 **</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>100</td>
<td>53.8 ± 2.7 **</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>5</td>
<td>90.6 ± 0.2</td>
<td>33.1 ± 1.0 *</td>
<td>27.1 ± 2.3</td>
<td>(-)</td>
</tr>
<tr>
<td>50</td>
<td>93.0 ± 0.8</td>
<td>22.1 ± 1.5 **</td>
<td>31.4 ± 3.2</td>
<td>(-)</td>
</tr>
<tr>
<td>100</td>
<td>80.7 ± 5.7</td>
<td>14.3 ± 0.7 ***</td>
<td>67.3 ± 1.5</td>
<td>(-)</td>
</tr>
<tr>
<td>6</td>
<td>94.8 ± 3.1</td>
<td>37.0 ± 1.8</td>
<td>18.5 ± 4.0</td>
<td>51.5 ± 0.5</td>
</tr>
<tr>
<td>25</td>
<td>93.7 ± 3.5</td>
<td>33.1 ± 2.4 *</td>
<td>27.1 ± 2.3</td>
<td>(-)</td>
</tr>
<tr>
<td>50</td>
<td>93.5 ± 3.0</td>
<td>22.9 ± 1.1 **</td>
<td>49.5 ± 2.4</td>
<td>(-)</td>
</tr>
<tr>
<td>100</td>
<td>81.3 ± 2.8</td>
<td>15.1 ± 0.3 ***</td>
<td>66.7 ± 0.6</td>
<td>(-)</td>
</tr>
<tr>
<td>7</td>
<td>96.6 ± 3.1</td>
<td>42.6 ± 0.7</td>
<td>62 ± 1.5</td>
<td>55.5 ± 0.3</td>
</tr>
<tr>
<td>25</td>
<td>93.6 ± 4.0</td>
<td>40.6 ± 2.3</td>
<td>10.6 ± 0.1</td>
<td>(-)</td>
</tr>
<tr>
<td>50</td>
<td>93.3 ± 2.0</td>
<td>22.7 ± 0.2 **</td>
<td>47.7 ± 0.3</td>
<td>(-)</td>
</tr>
<tr>
<td>100</td>
<td>83.7 ± 1.6</td>
<td>14.3 ± 0.3 ***</td>
<td>68.5 ± 0.7</td>
<td>(-)</td>
</tr>
<tr>
<td>8</td>
<td>95.4 ± 3.2</td>
<td>39.8 ± 1.2</td>
<td>12.3 ± 2.6</td>
<td>54.5 ± 0.8</td>
</tr>
<tr>
<td>25</td>
<td>94.9 ± 2.6</td>
<td>35.4 ± 0.5 *</td>
<td>21.9 ± 1.8</td>
<td>(-)</td>
</tr>
<tr>
<td>50</td>
<td>88.5 ± 2.6</td>
<td>22.5 ± 0.9 **</td>
<td>46.2 ± 2.0</td>
<td>(-)</td>
</tr>
<tr>
<td>100</td>
<td>87.4 ± 1.2</td>
<td>14.4 ± 0.3 ***</td>
<td>68.3 ± 0.7</td>
<td>(-)</td>
</tr>
</tbody>
</table>

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1H-NMR (CDCl3), chemical shift (ppm):  
- 0.40 (1H, d, J = 4.5 Hz, H-20), 1.31–1.43 (7H, m, H-1, -12, -26, -5, -17, -25)  
- 0.62 (1H, d, J = 4.5 Hz, H-20), 1.52–1.69 (9H, m, H-31, -11, -8, -15, -27, -6, -30, -16)  
- 0.78 (1H, m, H-6), 1.60 (3H, s, CH3-30), 1.86–2.05 (3H, m, H-23, -26, -7)  
- 0.80 (3H, d, J = 9.5 Hz, CH3-32), 3.01 (1H, d, J = 9.5 Hz, H-3)  
- 0.82 (3H, s, CH3-18), 3.15 (1H, d, J = 9.5 Hz, H-3), 3.21 (1H, m, H-7), 4.20 (1H, br s, H-29)  
- 0.91 (3H, s, CH3-21), 3.40 (1H, br s, OH), 4.64 (1H, br s, H-29)  
- 0.93 (3H, d, J = 6.0 Hz, CH3-33), 3.54 (1H, dd, J = 11.0, 6.0 Hz, H-24), 7.10 (1H, br d, J = 11.0 Hz, H-24), 4.64 (1H, br s, H-29)  
- 0.97 (3H, s, CH3-22), 3.64 (1H, br d, J = 16.0, 9.0 Hz, H-2), 7.10 (1H, br d, J = 11.0 Hz, H-24), 4.64 (1H, br s, H-29)  
- 0.98 (3H, s, CH3-19), 3.70 (1H, br d, J = 11.0 Hz, H-24), 4.64 (1H, br s, H-29)  
- 1.04 (1H, m, H-7), 1.10–1.20 (3H, m, H-1, -25, -16), 4.77 (1H, br s, H-29)

Chemical Constituents from the Leaves of *Annona reticulata* and Their Inhibitory Effects on NO Production

**Analisis Struktur**

**1H-NMR (CDCl3), chemical shift (ppm):**
- 0.40 (1H, d, J = 4.5 Hz, H-20), 1.31–1.43 (7H, m, H-1, -12, -26, -5, -17, -25), 0.62 (1H, d, J = 4.5 Hz, H-20), 1.52–1.69 (9H, m, H-31, -11, -8, -15, -27, -6, -30, -16), 0.78 (1H, m, H-6), 1.60 (3H, s, CH3-30), 1.86–2.05 (3H, m, H-23, -26, -7), 0.80 (3H, d, J = 9.5 Hz, CH3-32), 3.01 (1H, d, J = 9.5 Hz, H-3), 3.15 (1H, d, J = 9.5 Hz, H-3), 3.21 (1H, m, H-7), 4.20 (1H, br s, H-29), 4.64 (1H, br s, H-29), 0.93 (3H, d, J = 6.0 Hz, CH3-33), 3.54 (1H, dd, J = 11.0, 6.0 Hz, H-24), 7.10 (1H, br d, J = 11.0 Hz, H-24), 4.64 (1H, br s, H-29), 1.10–1.20 (3H, m, H-1, -25, -16), 4.77 (1H, br s, H-29)

**13C-NMR (CDCl3), chemical shift (ppm):**
- 15.1 (C-18), 18.1 (C-19), 19.0 (C-30), 19.2 (C-10), 19.3 (C-21), 20.7 (C-33), 20.9 (C-6), 21.3 (C-32), 25.1 (C-9), 25.7 (C-22), 25.7 (C-1), 26.5 (C-7), 26.8 (C-16), 27.3 (C-26), 27.7 (C-25), 29.7 (C-20), 30.2 (C-31), 31.8 (C-15), 35.3 (C-12), 39.7 (C-11), 40.3 (C-4), 43.1 (C-17), 45.0 (C-13), 46.2 (C-23), 47.1 (C-5), 47.7 (C-8), 48.7 (C-14), 55.4 (C-27), 62.8 (C-24), 71.0 (C-2), 83.2 (C-3), 111.9 (C-29), 147.4 (C-28)

ESI-MS m/z (rel. int.) 523 (M+Na)+, 100; HR-ESI-MS m/z 523.4122 [M+Na]+; (calcld for C33H56O3Na, 523.4127).

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**Prosedur ekstraksi dan isolasi**

Daun kering dari *Annona reticulata* L. (5.0 kg) ditumbuk halus dan direndam (maserasi) dalam metanol (20 L x 3) selama 24 jam pada suhu kamar. Kemudian disaring, filtrat yang diperoleh dipetakkan dengan rotari evaporator untuk menghasilkan crude ekstrak metanol seperti sirup berwarna coklat gelap (316.0 g). Ekstrak ini di dispersikan ke dalam air (1 L) dan dipartisi dengan n-heksan (1 L x 5), etil asetat (1 L x 5), n-butanol (1 L x 5). Masing-masing ekstrak hasil partisinya dilakukan evaporasi dihasilkan 31.0 g (ekstrak n-heksana), 82.0 g (etil asetat), 47.0 g (n-butanol), dan 52.0 g (air).

The *n*-hexane soluble extracts were purified by silica gel column chromatography eluted with *n*-hexane and acetone gradients to afford 14 fractions.

- **Fraction 1** was subjected to silica gel column chromatography eluted with *n*-hexane/acetone (25:1) to yield kaurenoic acid (2, 968 mg).
- **Fraction 2** was isolated by silica gel column chromatography eluted with *n*-hexane/acetone (15:1) to afford taraxerol (3, 78 mg).
- Purification of **fraction 3** by column chromatography with silica gel eluted by step gradients of *n*-hexane/acetone (15:1 and 9:1) afforded β-sitosterol (4, 302 mg).
- **Fraction 4** was subjected to silica gel column chromatography eluted with *n*-hexane/acetone (15:1) to yield 16α-hydro-19-al-ent-kauran-17-oic acid (5, 26 mg).
- **Fraction 5** was purified by silica gel column chromatography eluted with *n*-hexane/acetone (9:1) to yield 6β-hydroxystigmast-4-en-3-one (6, 31 mg).
- Isolation of **fraction 6** by column chromatography with silica gel eluted by *n*-hexane/acetone (7:1) yielded 17-acetoxy-16β-ent-kauran-19-oic acid (7, 22 mg).

Anti-cancer triterpenoid from sea cucumber

Triterpene glycosides, fuscocineroside A (100), B (101), and C (102), pervicoside C (103) and holothurin A (104) isolated from *Holothuria fuscocinerea* Jaeger

Active on human leukemia HL-60 and human hepatoma BEL-7402 cells.

All compounds showed a potent cytotoxicity towards both cell lines. However, fuscocineroside C (102) was found to be the most potent (IC50 = 0.88, IC50 = 0.58 μg/mL) in HL-60 and BEL-7402 cell lines.

IDENTIFICATION OF TRITERPENOIDE COMPOUND FROM \textit{Polyscias fruticosa} Harm. (Araliaceae) ROOT BARK

Extraction and isolation procedures:
Dried and milled of root bark of \textit{P. fruticosa} (3 kg) was extracted exhaustively with methanol at room temperature for 24 hours. Evaporated in reduced pressure of extract resulted brown residue (119 g) and suspended in water following extraction using n-hexane. A portion (20 g) of methanol extract was chromatographed by VLC eluted with n-butanol resulted 4 fractions. Major fraction was then separated by preparative TLC using eluent chloroform : methanol : water (2:6:1) and provided compound with R$_f$ 0.55, 0.64 and 0.73.

Structure Analysis
MS
$^1$H- and $^{13}$C-NMR
FTIR Spectrophotometry
UV-Vis Spectrophotometry

Karakterisasi Senyawa Triterpenoid dari Kulit Batang Tanaman Angsret, *Spathodea campanulata* P. Beauv (Bignonaceae)


Gambar 6 Pengaruh konsentrasi triterpenoid terhadap pertumbuhan *E. coli*.

Gambar 7 Pengaruh konsentrasi triterpenoid terhadap pertumbuhan *S. aureus*.
2.2 Prosedur Ekstraksi dan Isolasi

Sebanyak 1,2 kg sampel kering dibagi per 150 gram direndam dengan 250 mL (total 4800 mL metanol) selama 24 jam. Perlakuan perendaman dilakukan sebanyak 7 kali ulangan. Ekstrak disaring dengan kertas saring sehingga diperoleh filtrat yang kemudian dipetakkan dengan rotary evaporator vacuum. Ekstrak pekat sebanyak 100 mL dimasukkan dalam corong pisah 500 mL dan diekstraksi dengan heksana, dikocok dan dibiarkan sampai terbentuk dua lapisan. Lapisan heksana diambil dan dipetakkan dengan rotary evaporator vacuum. Selanjutnya fraksi n-heksan dipisahkan dengan kromatografi kolom dengan eluen n-heksan/etil asetat (10/80). Dari kolom ini diperoleh fraksi dengan karakter triterpenoid dan aktif sebagai antibakteri. Kemudian dianalisis dengan metode spektrometri.

Phytochemicals screening test of triterpenoid dan steroid

Triterpenoid and steroid test was conducted according to Lieberman-Burchard1 and Salkowski test2:

The Lieberman-Burchard test1 was performed by addition of sample (2 mL) with a few drops of concentrated sulphuric acid and acetic anhydride. The presence of terpenoid was indicated with color blue, red or violet.

Salkowski test2 was performed by addition of methanol extract sample (2 mL) with chloroform (1 mL), and concentrated sulphuric acid (1 mL). The presence of red or orange indicated triterpenoid steroid compounds.