# EVALUATION OF LIMONOIDS AGAINST THE MURINE P388 LYMPHOCYTIC LEUKEMIA CELL LINE[1]

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ABSTRACT.—A structure/activity study of selected limonoids from two plant families (Meliaceae and Rutaceae) of the Rutales order against the murine P-388 lymphocytic leukemia system was undertaken. The presence of both a 19↦28 lactol and a 14,15β-epoxide group was found especially important for pronounced inhibition of the PS *in vitro* cell line. Substitution of an A-ring α,β-unsaturated ketone (3-oxo-1-ene) for the lactol led to diminished activity, while reduction of the olefin caused complete loss of activity. At the dose levels employed, even very good PS *in vitro* inhibition was not translated into PS *in vivo* antineoplastic effects.

A number of Meliaceae species are well-known medicinal plants (2). And 14, such as *Amoora rohituka* (India), *Khaya ivorensis* (Nigeria), *Melia azedarach* L. (Columbia), *Sandoricum indicum* (Mexico), and *Trichilia hirta* L. (Venezuela), have been used in the primitive treatment of cancer (2). In our evaluation of the Meliaceae family (Rutales order) as a source for potentially useful antineoplastic agents, we have uncovered a series of tetranortriterpenes that strongly inhibit growth of the National Cancer Institute's murine P-388 lymphocytic leukemia (PS system) cell line. Representative examples include amoorastatin (**3**) from seeds of the Eastern Himalayan *Aphanamixis grandifolia* Bl. (3,4) and 14,15β-epoxy-prieurianin (**24**) from root bark of the French Guiana medicinal tree *Guarea guidona* L. Sleumer (5). In addition, three cytotoxic (KB) limonoids recently have been isolated from *Trichilia hispida* (6).

In order to better define PS *in vitro* structure/activity relationships among limonoids with the prospect of possibly locating requirements for *in vivo* antineoplastic effects, we have evaluated a number of such substances from the Meliaceae and Rutaceae (Rutales order) families (table 1). As expected, most of the interesting leads were discovered by bioassay-directed isolation (*e.g.*, **3,4,5**) rather than by exploratory evaluation of limonoids originally uncovered for other purposes. But the latter specimens did prove very useful in helping to further define structural features necessary for *in vitro* activity.

Inspection of the PS *in vitro* data (7) summarized in table 1 revealed that, among the tetranortriterpenes with intact A-D ring systems, presence of the 19↦28 lactol and 14,15β-epoxide groups seems essential for the marked cell growth inhibition displayed by compounds **2-5**. Earlier, the furan ring was also considered important (4), but, as noted in the sequel, this may be only a minor structural requirement. Replacement of the hemiacetal by a 3-oxo-1-ene system as evidenced by ketones **8-10** (and **13**) allowed some activity, while related A-ring saturated derivatives such as **6** and **7** were inactive. An analogous correlation was seen with the 16a-oxa-tetranortriterpenes **17** and **18**. And utility of the 7α-hydroxyl group for PS *in vitro* activity was evidenced by the inactivity of 7-oxo-gedunin (**16**). The 3a-oxa-tetranortriterpenes **19** and **20** were found inactive. But two of the 3a-oxa-terpenes with a *seco*-ring C (**24** and **25**), isolated by bioassay, proved to have some inhibitory activity against the PS *in vitro* cell line.

To further assess importance of the A-ring a series of *citrus* limonoids (**28-38**)[4] was studied. While all were found inactive, they did serve to emphasize importance of the A-ring molecular geometry displayed by, *e.g.*, deacetylgedunin (**17**). The 3-oxo-1-ene

[1]Antineoplastic Agents 87; for part 86 refer to (1).

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[4]For a recent advance in the area of Rutaceae chemistry consult (9).

TABLE 1. Rutales order: limonoids.

Meliaceae

**1** Amoorastatone (4)
*Aphanamixis grandifolia* Bl.
P-388: $ED_{50}$=30
T/C inactive (2.4↦0.01 mg)

**2** 12-Hydroxyamoorastatin (4)
*Aphanamixis grandifolia* Bl.
P-388: $ED_{50}$=0.002
T/C toxic (20↦5 mg)
inactive (2.5↦1.25 mg)

**3** Amoorastatin (3)
*Aphanamixis grandifolia* Bl.
P-388: $ED_{50}$<0.001
T/C inactive (2↦0.25 mg)

**4** Sendanin (4)
*Melia azedarach* Linn.
P-388: $ED_{50}$<0.01
T/C inactive (1↦0.13 mg)

**5** Aphanastatin (8)
*Aphanamixis grandifolia* Bl.
P-388: $ED_{50}$=0.04

**6** Substance Tb2 (14)
*Turrea floribunda*
P-388: $ED_{50}$>100

**7** Substance Tb3 (14)
*Turrea floribunda*
P-388: $ED_{50}$=40

**8** Substance Tb2ox (14)
*Turrea floribunda*
P-388: $ED_{50}$=3.0
T/C inactive (12↦0.75 mg)

**9** Substance Tb3ox (14)
*Turrea floribunda*
P-388: $ED_{50}$=2.8
T/C inactive (12↦0.75 mg)

**10** Anthothecol (15)
*Khaya anthotheca* D.C.
P-388: $ED_{50}$=1.2
T/C inactive (24↦3 mg)

**11** Melianone (16,45)
*Melia azedarach* L.; *Simarouba amara*
P-388: $ED_{50}$=24
T/C inactive (12↦1.5 mg)

**12** 1β,2β-Diepoxyazadiradione (17)
*Azadirachta indica*
P-388: $ED_{50}$>10

**13** 7-Acetylneotrichilenone (17)
*Azadirachta indica*
P-388: $ED_{50}$=8.5

**14** Deacetyldihydrogedunin (18,19)
*Guarea thompsonii*
P-388: $ED_{50}$>10

**15** 7-Ketodihydrogedunin (19)
*Guarea thompsonii*
P-388: $ED_{50}$>10
T/C inactive (8↦0.5 mg)

**16** 7-Oxogedunin (18)
*Carapa guayanensis*
P-388: $ED_{50}$>100
T/C inactive (24↦1.5 mg)

**17** Deacetylgedunin (20)
*Entandrophragma angolense*
P-388: $ED_{50}$=0.11
T/C inactive (24↦1.5 mg)

**18** 14,15-Deoxygedunin (21)
*Entandrophragma angolense*
P-388: $ED_{50}$=11
T/C inactive (40↦2.5 mg)

**19** Surenone (22)
*Toona sureni* (Blume) Merill
P-388: $ED_{50}$>100
T/C inactive (10↦1.25 mg)

**20** Surenin (22)
*Toona sureni* (Blume) Merill
P-388: $ED_{50}$>10
T/C inactive (10↦1.25 mg)

**21** Ekebergolactone I (14)
*Ekebergia senegalensis*
P-388: $ED_{50}$>10

**22** Methyl angolensate (23,24)
*Entandrophragma angolense*
P-388: $ED_{50}$>10
T/C inactive (200↦2 mg)

**23** Dregeanin (25)
*Trichilia dregeana*
P-388: $ED_{50}$>10

**24** 14,15β-Epoxyprieurianin (5)
*Guarea guidona* L.
P-388: $ED_{50}$=4.5

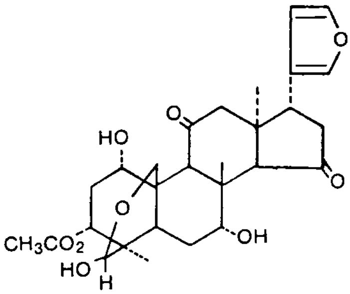
TABLE 1. Continued.

| Meliaceae | |
|---|---|
| **25** Prieurianin (26,27)<br>*Guarea guidona* L.; *Trichilia prieuriana*<br>P-388: $ED_{50}$=4.4<br>T/C toxic (15 mg)<br>inactive (7.5↦3.7 mg) | **26** Substance B (14)<br>*Guarea thompsonii*<br>P-388: $ED_{50}$>100 |
| **27** Rohitukin (28)<br>*Aphanamixis rohituka*<br>P-388: $ED_{50}$=100<br>T/C inactive (2↦0.25 mg) | |
| **Rutaceae** | |
| **28** Limonin (29)<br>*Citrus* sp. (seeds)<br>P-388: $ED_{50}$>100 | **29** Desoxylimonin (30)<br>*Citrus* sp. (seeds)<br>P-388: $ED_{50}$>10<br>T/C inactive (4↦0.5 mg) |
| **30** Limonin diosphenol (29,31)<br>*Evodia* sp.<br>P-388: $ED_{50}$>10<br>T/C toxic (8 mg)<br>inactive (4↦0.5 mg) | **31** Limonin diosphenol acetate (29)<br>P-388: $ED_{50}$>10<br>T/C toxic (8 mg)<br>inactive (4↦0.5 mg) |
| **32** Limonilic acid (32)<br>*Citrus* sp. (seeds)<br>P-388: $ED_{50}$>10<br>T/C inactive (6↦0.75 mg) | **33** Desoxylimonic acid (29)<br>P-388: $ED_{50}$>10<br>T/C inactive (4↦0.5 mg) |
| **34** Tetrahydrolimonilic acid (32)<br>P-388: $ED_{50}$>10<br>T/C toxic (8 mg)<br>inactive (4↦0.5 mg) | **35** Obacunoic acid (33)<br>*Dictamnus* sp.<br>P-388: $ED_{50}$>10<br>T/C inactive (6↦0.75 mg) |
| **36** Nomilin (34)<br>*Citrus* sp. (seeds)<br>P-388: $ED_{50}$>10 | **37** Epilimonol chloroacetate (29)<br>P-388: $ED_{50}$>10<br>T/C inactive (8↦0.5 mg) |
| **38** Epilimonol (29,35)<br>P-388: $ED_{50}$>10<br>T/C inactive (6↦0.75 mg) | |

groups of furan **17** and related substances might be necessary for inhibiting growth of the PS cell line by interfering with certain necessary thiols and/or amines through Michael-type additions reactions. Possibly the 14,15β-epoxide is required for concurrent alkylation[5] of such biopolymer amine and/or thiol sites, while the furan ring and hydroxyl substituents simply assist in transport.

Some indication that the D-ring lactone and furan ring systems are not essential for retarding growth of the PS cell line was obtained by evaluating a series (**39-50**) of naturally occurring furans (table 2) found in several other plant families. All (including

[5]We have observed an analogous situation with PS *in vitro* active pseudoguaianolides such as autumnolide, and the need for two α,β-unsaturated systems with related substances such as helenalin and radiatin. (10,11).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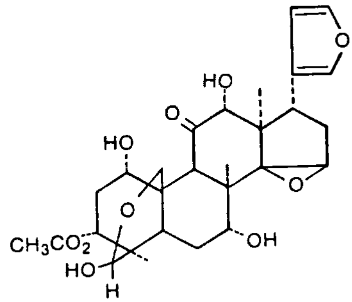
1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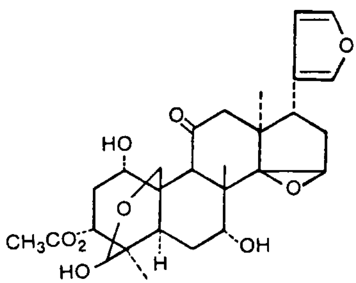
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OH
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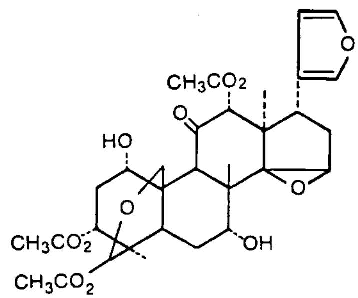
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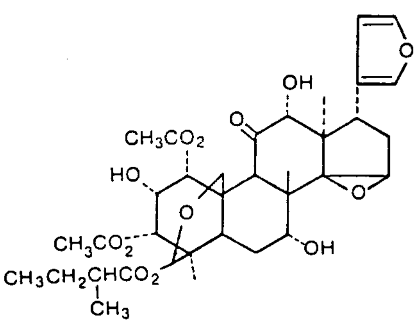
CH3CO2

4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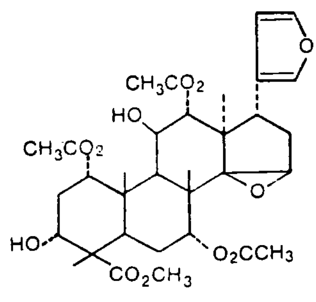
5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HO
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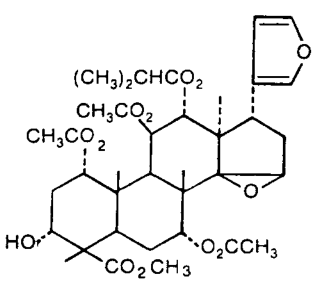
CO2CH3

6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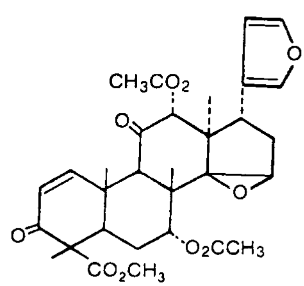
CO2CH3

7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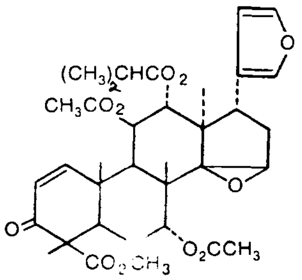


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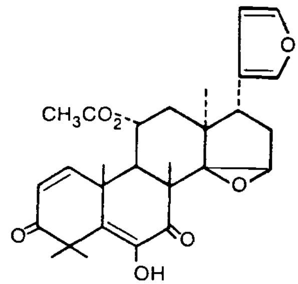


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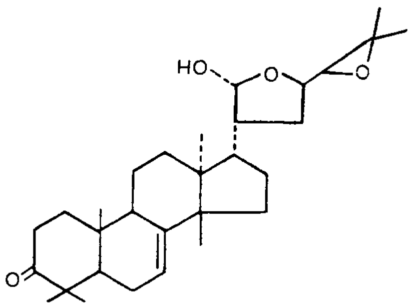


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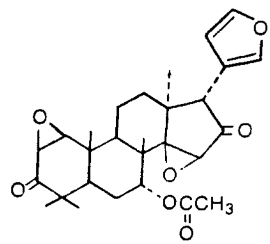


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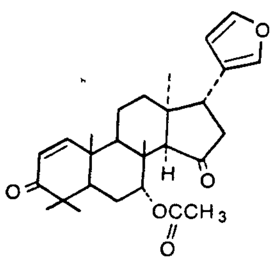


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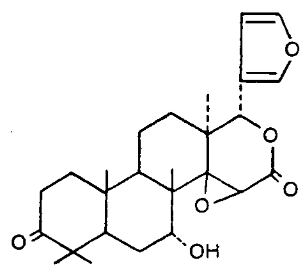


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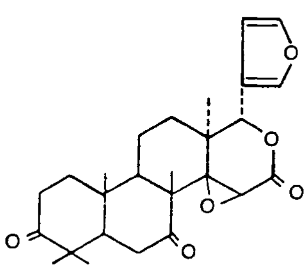


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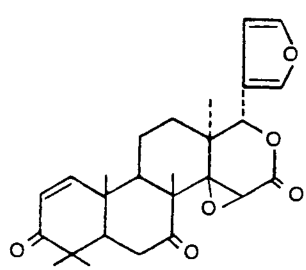


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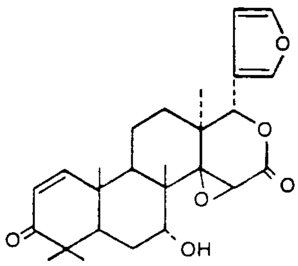


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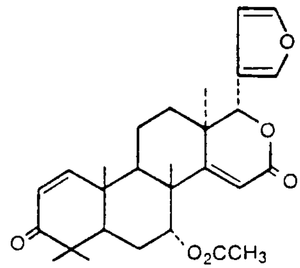


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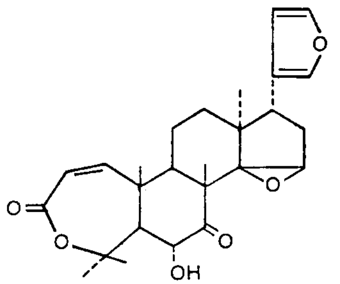


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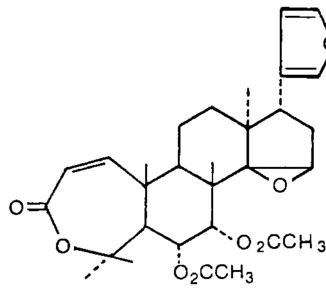


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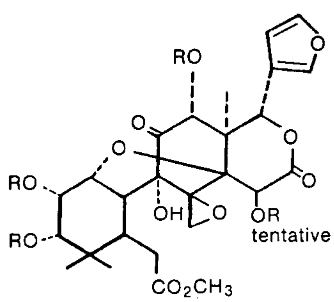
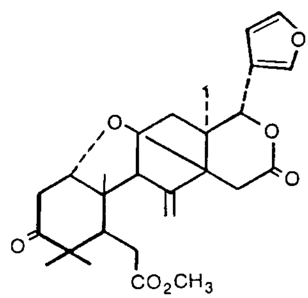



**21** R = $CH_3CO$, $CH_3CO$
$(CH_3)_2CHO$,
$CH_3CH_2(CH_3)CHCO$

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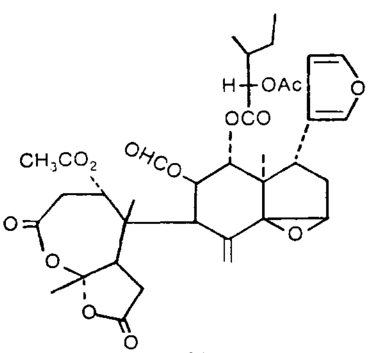


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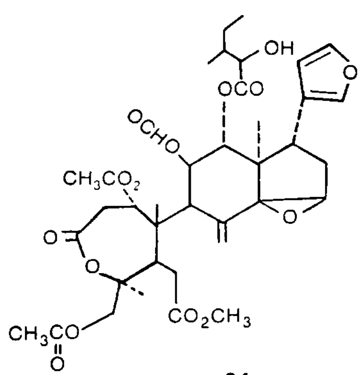


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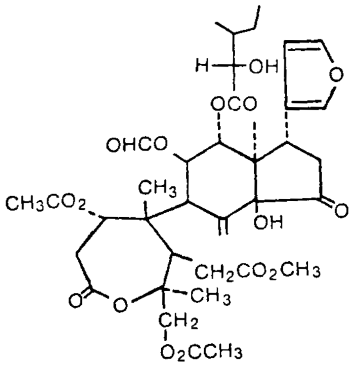


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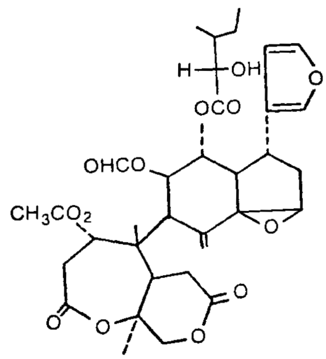


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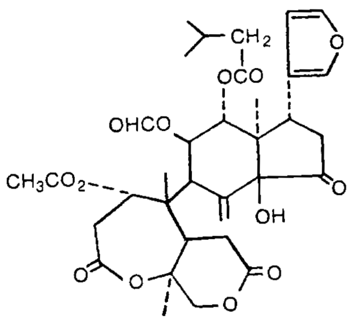


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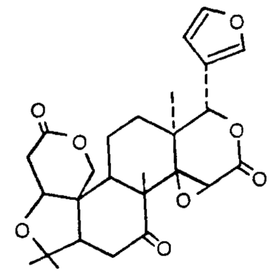

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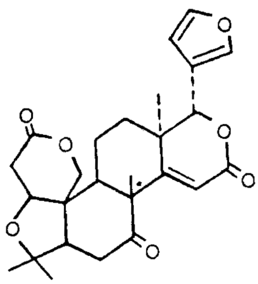

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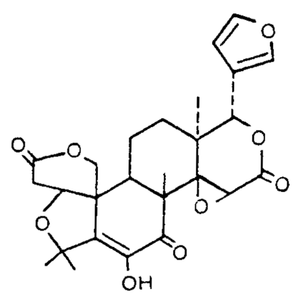


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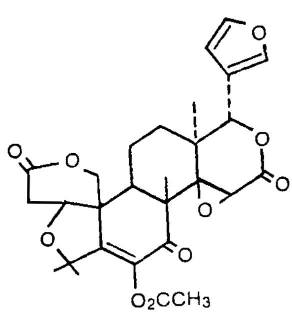


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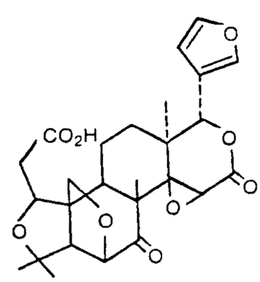


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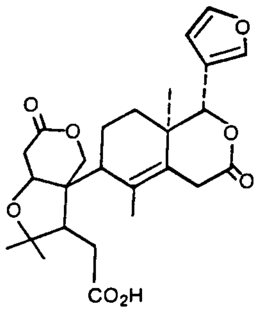


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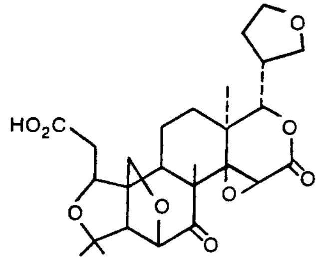


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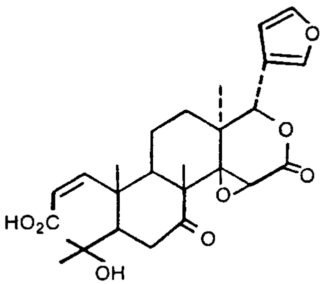


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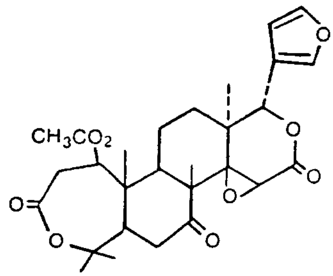


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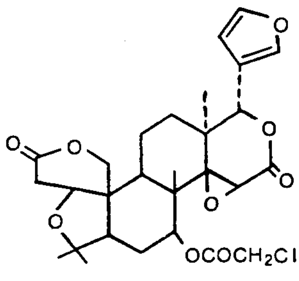


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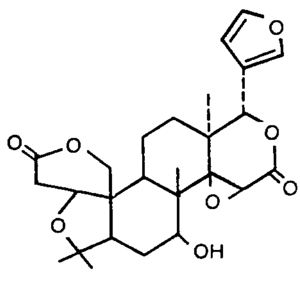


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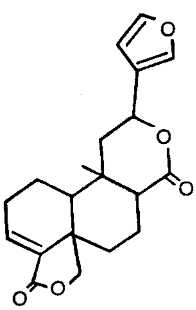

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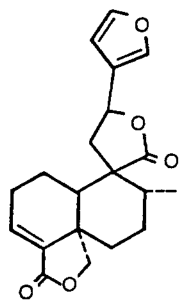

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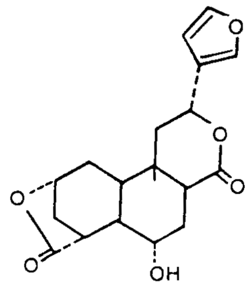

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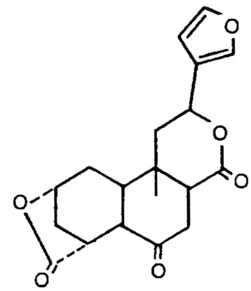

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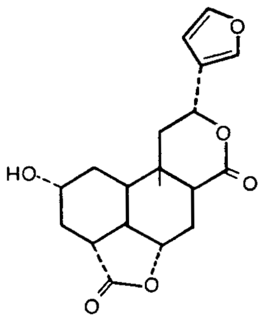

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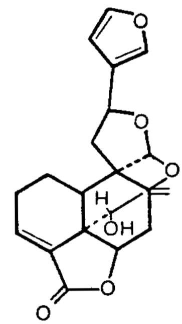

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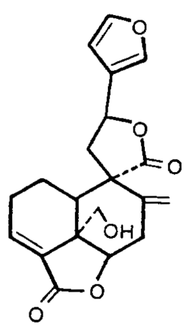

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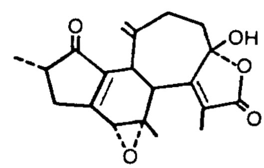

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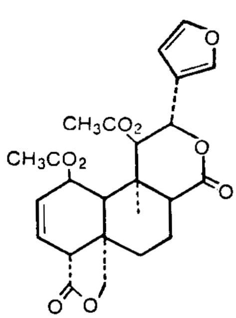

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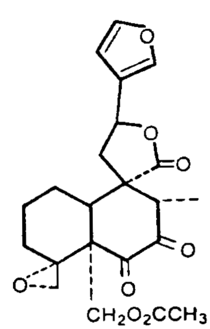

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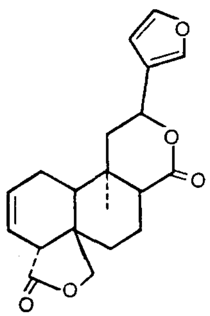

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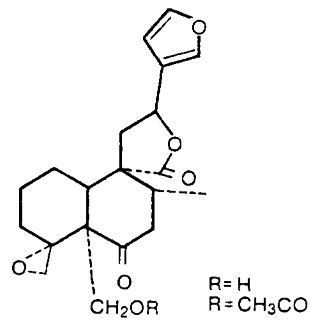


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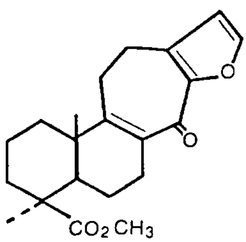


51

TABLE 2. Naturally occurring furans.

Asteraceae

**39** Bacchotricuneatin A (36)
*Baccharis tricuneata*
P-388: $ED_{50}>100$
T/C inactive (2↦0.25 mg)

**40** Bacchotricuneatin B (36)
*Baccharis tricuneata*
P-388: $ED_{50}>100$
T/C inactive (2↦0.03 mg)

**41** Diosbulbin E (37)
*Dioscorea bulbifera* L.
P-388: $ED_{50}>10$

**42** Diosbulbin D (37)
*Dioscorea bulbifera* L.
P-388: $ED_{50}>10$

**43** Diosbulbin G (37)
*Dioscorea bulbifera* L.
P-388: $ED_{50}>10$

Euphorbiaceae

**44** Plaunol A (38)
*Croton sublyratus* Kurz
P-388: $ED_{50}>10$

**45** Plaunol B (38)
*Croton sublyratus* Kurz
P-388: $ED_{50}=2.1$

**46** Crotofolin E (39)
*Croton corylifolius* L.
P-388: $ED_{50}>10$

**47** Splendidin (40)
*Salvia splendens*
P-388: $ED_{50}>10$

**48** Clerodane-6,7-dione (41)
*Teucrium polium* L.
P-388: $ED_{50}>10$

**49** Salviarin (42)
*Salvia splendens*
P-388: $ED_{50}>10$

**50** Gnapholin, R=H
19-Acetylgnaphalin, R=$CH_3CO$ (43)
*Teucrium gnaphalodes*
P-388: $ED_{50}>10$, both compounds

**51** Methyl hispanonate (44)
*Ballota hispanica* Neck ex Nim.
P-388: $ED_{50}=5.6$
T/C inactive (6↦0.37 mg)

butenolide **46**), except the unusual fused-ring furan methyl hispanonate (**51**), were found to be inactive.

A majority of the substances summarized in tables 1 and 2 were used against the murine PS *in vivo* screen at the highest doses permitted by the availability of each compound. Interestingly, all gave inactive results, suggesting the necessity of higher dose levels or activity limited to the cell line.[6] Unless eventual higher dose level studies (PS and some solid tumors) prove fruitful, the sample of limonoid bitter principles so far studied suggests that this interesting class of degraded (C-26) (12) triterpenes may not necessarily be responsible for the PS *in vivo* activity shown by some Meliaceae species. Attention will now be redirected to other types of constituents from such potentially important plants.

## METHODS

The PS *in vitro* studies were conducted in our laboratory employing a culture of P-388 murine lymphoblastoid cells (7) obtained from the National Cancer Institute (NCI). Experimental and control procedures developed by the NCI were used for each evaluation. The PS *in vivo* bioassays were prepared under auspices of the NCI, as previously described (13).

## ACKNOWLEDGMENTS

The ASU-CRI and ICSN groups are very pleased to acknowledge support of this investigation by grants CA 26699-02 and CA 16049-04-07 awarded by the National Cancer Institute, DHW, and contract NO1-CM-97297 with the Division of Cancer Treatment, NCI, Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), Eleanor W. Libby, The Waddell Foundation (David Ware), Mr. Robert B. Dalton, Pearl Spear, and the Fannie E. Rippel Foundation.

We also wish to extend our appreciation and thanks to Drs. E. O. Arene, G. M. Cragg, T. Komori, W. Kraus, P. S. Manchand, A. Ogiso, H. Wagner, C. Marguez, F. Piozzi, G. Savona, and B. Rodriguez for assistance with some of the authentic specimens required for the study.

*Received 17 August 1982*

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[6]Generally we have found a very high predictive correlation between PS *in vitro* activity and subsequent PS *in vivo* results.

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