

SUMMARY OF DATA FOR CHEMICAL SELECTION

Apigenin

520-36-5

BASIS OF NOMINATION TO THE CSWG

Apigenin is brought to the attention of the CSWG because of a recent scientific article citing this flavonoid as a substance that can be metabolically activated to produce toxic prooxidant phenoxy radicals.

Pure apigenin is used primarily in research as a protein kinase inhibitor that may suppress tumor promotion and that has anti-proliferating effects on human breast cancer cells and inhibitory actions on MAP kinase. Apigenin is also one of several active ingredients in the popular herbal remedy, chamomile. Apigenin is found naturally in many fruits and vegetables, including apples and celery. It is found in several popular spices, including basil, oregano, tarragon, cilantro, and parsley.

As a representative of flavonoids containing phenol B rings that may induce lipid peroxidation, apigenin is a candidate for testing.

SELECTION STATUS

ACTION BY CSWG: 12/12/00

Studies requested:

Developmental toxicity

Short-term tests for chromosomal aberrations

Priority: None assigned

Rationale/Remarks:

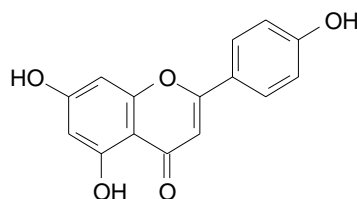
Nomination based on concerns about apigenin's potential to produce possibly toxic radicals and its estrogenic activity

NCI will conduct a mouse lymphoma assay

CHEMICAL IDENTIFICATION

<u>CAS Registry Number:</u>	520-36-5
<u>Chemical Abstracts Service Name:</u>	4H-1-benzopyran-4-one,5,7-dihydroxy-2-(4-hydroxy-phenyl)- (9CI)
<u>Synonyms and Trade Names:</u>	Apigenin; apigenine; apigenol; chamomile; C.I. natural yellow 1; 2-(<i>p</i> -hydroxyphenyl)-5,7-dihydroxy-chromone; spigenin; 4',5,7-trihydroxyflavone
<u>Structural Class:</u>	Flavone

Structure, Molecular Formula and Molecular Weight:



C₁₅H₁₀O₅

Mol. wt.: 270.24

Chemical and Physical Properties:

<u>Description:</u>	Yellow needles (Budavari, 1997)
<u>Melting Point:</u>	347.5°C (Lide, 1997)
<u>Solubility:</u>	Practically insoluble in water, moderately soluble in hot alcohol, soluble in dilute KOH (Budavari, 1997)
<u>Reactivity:</u>	Incompatible with strong oxidizing agents (Sigma-Aldrich, 2000)

Technical Products and Impurities: Apigenin is available at 95+% purity from Sigma-Aldrich and at an unspecified purity from Fisher Scientific (Fisher Scientific, 2000; Sigma-Aldrich, 2000).

EXPOSURE INFORMATION

Production and Producers:

Manufacturing process. An industrially significant extractive process for producing very pure apigenin has been described in the patent literature. The ligules of *Matricaria chamomilla* L. are extracted continuously for 7-8 hours with a water/ethanol mixture. The extract is evaporated to dryness, taken up in ether, and agitated for 24 hours at ambient temperature. The precipitate is hydrolyzed with 10% hydrochloric acid by heating under reflux for about 10 hours by which time all the apigenin precipitates as free aglycone. The apigenin can be further purified by crystallization from ethanol or other suitable solvents (Redaelli, 1982).

Producers and importers. Eleven US producers or distributors of apigenin are listed by Chem-Sources USA (Chemical Sources International, 2000). Sources of apigenin identified from chemical catalogs include: A.G. Scientific, Inc.; Aldrich; Fisher Scientific; Fluka; Indofine Chemical Company, Inc.; Sigma, and Tocris (A.G. Scientific, 2000; Block, 2000; Fisher Scientific, 2000; Sigma-Aldrich, 2000; Tocris, 2000).

Production/import/export level. Apigenin is not listed in EPA's Toxic Substances Control Act (TSCA) Inventory (NLM, 2000a).

Use Pattern: Pure apigenin is used as a research chemical (A.G. Scientific, 2000; Tocris, 2000).

According to the US Patent and Trademark Office (USPTO), 107 patents involving apigenin in some manner have been obtained in the United States. These patents involve a variety of uses including the preparation of antiviral agents for the treatment of HIV and other infections, particle binders, and pharmaceuticals for treatment of diseases including inflammatory bowel disease and skin conditions (USPTO, 2000).

Apigenin is recognized in traditional or alternative medicine for its pharmacological activity. For this reason, passion flower, which is rich in apigenin, has been used for treating intransigent insomnia, as an anti-spasmodic in Parkinson's disease and asthma, to reduce nerve pain in neuralgia, and to treat shingles (Hoffman, 2000a).

Apigenin is a major constituent of chamomile, which is recognized for its antiphlogistic, antispasmodic, and antibacterial effects. Chamomile tea (3-4 cups a day) has been used for centuries as a folk remedy for relieving indigestion or calming gastritis. Common alternatives are 2-3 grams of the herb in capsule form or 4-6 ml of tincture three times per day. Chamomile preparations are also widely used in skin care products to reduce cutaneous inflammation and other dermatological diseases (Gruenwald *et al.*, 1998; Healthnotes, 1999; Hoffman, 2000b; Nemezc, 2000).

There are actually two herbs called chamomile, Roman chamomile (*Chamaemelum nobile*, *Anthemis nobilis*) and German chamomile (*Matricaria recutita*, *Chamomilla recutita*). German chamomile is more frequently preferred for medicinal use. *Chamomilla recutita* contains 0.24-1.9% volatile oil which contains up to 50% α -bisabolol, 1-15% chamazulene, and bisabolol oxides. German chamomile contains flavonoids (up to 8%), including apigenin and luteolin. Apigenin is also found in Roman chamomile. Apigenin and apigenin 7-*O*-glucoside have been shown to penetrate into deeper skin layers when applied topically which supports the use of chamomile as a topical antiphlogistic agent to treat inflammations in deep tissues (Merfort *et al.*, 1994; Nemezc, 2000; Webmd.com, 2000).

Human Exposure: No reports of occupational exposure to apigenin during its production or processing were found in the available literature. No listing was found for apigenin in the National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983.

Human exposure to apigenin occurs primarily through the consumption of chamomile and through its presence as a glycoside in many fruits and vegetables. These glycosides are efficiently hydrolyzed *in vivo* by bacterial enzymes in the human intestinal tract to the free flavonoids (Eaton *et al.*, 1996).

Foods rich in apigenin include apples, endive, beans, broccoli, celery, cherries, cloves, grapes, leeks, onions, barley, parsley and tomatoes, while plant-derived beverages containing apigenin include tea and wine (Janssen *et al.*, 1998).

Environmental Occurrence: Apigenin is natural flavonoid present in the leaves and stems of vascular plants, including fruits and vegetables (Lepley *et al.*, 1996).

Common plant species with the highest amounts of apigenin include:

- Achillea millefolium* L. – yarrow, in plant
- Apium graveolens* L. - celery, in plant
- Artemisia dracunculus* L. - tarragon, in plant
- Camellia sinensis* (L.) - tea, in leaf
- Chamaemelum nobile* (L.) - perennial chamomile, in plant
- Coriandrum sativum* L. - cilantro, in fruit
- Digitalis purpurea* L. - purple foxglove, in flower
- Echinacea spp* - coneflower, in leaf
- Ginkgo biloba* L. - in leaf
- Glycyrrhiza glabra* L. - licorice, in root
- Linum usitatissimum* L. - flax, in plant
- Marrubium vulgare* L. - horehound, in plant
- Matricaria recutita* L.- annual chamomile, in plant
- Mentha spicata* L. - spearmint, in leaf
- Ocimum basilicum* L. - basil, in plant
- Origanum vulgare* L. - oregano, in plant (Duke & Beckstrom-Sternberg,

2000).

Regulatory Status: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of apigenin. Apigenin was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) or biological exposure index (BEI) are made.

Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA). The DSHEA requires no proof of safety for dietary supplements on the market prior to October 15, 1994. Labeling requirements for dietary supplements allow warnings and dosage recommendations as well as substantiated “structure or function” claims. All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement “This product is not intended to diagnose, treat, cure, or prevent any disease” (FDA, 1995).

In Germany, chamomile flower is licensed as a medicinal tea (infusion) for oral ingestion, for topical application as a rinse or gargle, cream, or ointment, as a vapor inhalant, and as a bath additive. German chamomile is classified in the *Homeopathic Pharmacopoeia of the United States* as an over-the-counter Class C drug prepared as a 1:10 (w/v) alcoholic tincture of the whole flowering plant, in 45% v/v alcohol (Webmd.com, 2000).

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to apigenin and cancer risk in humans were identified in the available literature.

Animal Data: No 2-year carcinogenicity studies of apigenin were identified in the available literature. The only acute toxicity value found in the available literature reported an LD₂₅ of 1 mg/kg when apigenin 8-C-glucoside was administered to mice via intraperitoneal injection (NLM, 1999).

Short-Term Tests: In the reverse mutation assay, apigenin was negative in the *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535, and TA1538 with or without metabolic activation by the S-9 microsomal enzyme fraction (NLM, 2000b).

The mutagenic activity of apigenin in *Escherichia coli* has also been examined. Apigenin weakly induced the SOS repair system in *E. coli* K-12 strain PQ 37 with and without metabolic activation (Czczot & Bilbin, 1991).

Metabolism:

Human Data. Apigenin appears to be absorbable by humans after intake of parsley (*Petroselinum crispum*). In a randomized crossover study with two one-week intervention periods in succession, fourteen volunteers consumed a diet that included 20 g parsley. The urinary excretion of apigenin was significantly higher ($P < 0.05$) during the intervention with parsley (20.7 – 5727.3 g/24 hr) than during the basic diet (0 – 1571.7 g/24 hr). The half-life for apigenin was calculated to be on the order of 12 hr. Significant individual variation in the bioavailability and excretion of apigenin was observed (Nielsen *et al.*, 1999).

Apigenin derived from aqueous alcoholic extracts of chamomile [*Chamomilla recutita*] flower heads concentrated in the stratum corneum within the first two hours of dermal exposure in human subjects. After three hours, a steady state

was attained, suggesting that apigenin diffused through deeper skin layers to be absorbed afterwards by cutaneous blood and lymph vessels (Merfort *et al.*, 1994).

Animal Studies. Ether extracts of the urine of male Wistar rats administered apigenin (200 mg) orally contained the phenolic acid metabolites *p*-hydroxyphenylpropionic acid, *p*-hydroxycinnamic acid, and *p*-hydroxybenzoic acid. Unreacted apigenin, partially characterized apigenin glucuronides, and ethereal sulfates were also identified. With the exception of *p*-hydroxybenzoic acid and the apigenin conjugates, all of the metabolites detected in the urine after oral administration were also formed *in vitro* by rat intestinal microorganisms under anaerobic conditions (Griffiths & Smith, 1972).

In contrast, these metabolites were not detected in SENCAR mice treated topically with apigenin. Furthermore, no evidence of metabolites were observed from the HPLC profiles of epidermal extracts from apigenin-treated mice (Li *et al.*, 1996).

Four hours after administration of a flavonoid glycoside extract (corresponding to 0.942 mg aglycones) by gavage, the aglycone of apigenin was observed in the lumen and the wall of the stomach, in the lumen of the small intestine and in the lumen and wall of the cecum in Wistar rats. The evidence of glycosides in the stomach wall suggested that the absorption of flavonoids did not require the presence of their aglycones. Under the study conditions, no renal excretion of apigenin was detected (Pforte *et al.*, 1999).

In Vitro Studies. The main *in vitro* metabolite of apigenin in rat liver Aroclor 1254-induced microsomes has been identified tentatively as the corresponding 3'-hydroxylated compound, luteolin. Apigenin itself is the 3'-hydroxylated metabolite of chrysin (Nielsen *et al.*, 1998).

In Hep G2 hepatic cells, only two apigenin metabolites, identified as glucuronic acid and sulfate conjugates, were detected. These observations and the observations in animals discussed earlier, emphasize the importance of phase II

conjugation reactions in the metabolism of flavonoids and suggest that both sulfation and glucuronidation are critical determinants of the oral bioavailability of flavonoids in humans, although a contribution from CYP-mediated oxidation cannot be excluded (Galijatovic *et al.*, 1999).

Other Biological Effects: *Anticarcinogenic Activity.* Topical administration of apigenin inhibited dimethylbenzanthracene (DMBA)-induced skin tumors in Swiss mice (PHS-149, 1972). In the SKH-1 mouse, pretreatment with topical apigenin resulted in reduction in UVB-induced cancer incidence (52% inhibition) and an increase in tumor-free survival ($P < 0.01$). In contrast, apigenin did not prevent the *in vitro* production of photoproducts in salmon sperm DNA, suggesting that apigenin did not inhibit UVB carcinogenesis by simply absorbing ultraviolet light or decreasing DNA damage (Birt *et al.*, 1997).

Inhibition of Tumor Promotion. When given topically 30 minutes prior to 12-*O*-tetradecanoylphorbol-13-acetate (TPA), apigenin in dimethylsulfoxide (DMSO) reduced the incidence and multiplicity of papillomas and carcinomas in DMBA-initiated SENCAR mouse skin, prolonged the latency period of tumor appearance, and showed the tendency to decrease conversion of papillomas to carcinomas (Wei *et al.*, 1990). However, in subsequent experiments, topical treatment with apigenin in acetone/DMSO prior to TPA did not inhibit skin carcinogenesis in SENCAR mice. The differences in results were attributed to effects of the vehicles (Li *et al.*, 1996).

Apigenin has also been shown to counteract tumor promoter-induced inhibition of intercellular communication in rat liver epithelium. When exposed to 25 μM apigenin and either TPA or butylated hydroxytoluene (BHT), rat liver epithelial cells exhibited an increase in gap junctional intercellular communication (GJIC), thereby inhibiting the GJIC inhibition induced by the tumor promoters (Chaumontet *et al.*, 1997).

Enzyme Inhibition. Antitumorogenic properties of apigenin have been attributed to its ability to inhibit chemically induced ornithine decarboxylase

(ODC) activity. Specifically, apigenin in DMSO inhibited ODC activity in SENCAR mice in a dose-dependent manner (Wei *et al.*, 1990). When administered in acetone/DMSO, however, apigenin was not as effective in ODC inhibition (Li *et al.*, 1996).

Le Bail and co-workers showed that apigenin was an effective inhibitor of aromatase (human estrogen synthetase) and 17 α -hydroxysteroid dehydrogenase activities in human placental microsomes, suggesting that it may be beneficial in treatment of human breast cancer (Jeong *et al.*, 1999; Le Bail *et al.*, 1998).

Molecular Signaling Effects. Apigenin significantly inhibited TPA-induced proto-oncogene *c-jun* activation in NIH Swiss NIH3T3 mouse embryonic cells; protein kinase C (PKC) activity in NIH3T3 cells; transformation (as determined by formation of foci) of C3H1 mouse embryonic fibroblasts, and *H-ras*-transformant growth of NIH3T3 cells (Lee & Lin, 1997; Lin *et al.*, 1997).

As a phosphatidylinositol 3-kinase (PI3K) inhibitor, apigenin blocked peroxisome proliferator-induced phosphorylation of the extracellular signal-regulated kinase (ERK), a mitogen-activated protein kinase (MAPK), in freshly isolated hepatocytes from B6C3F1 mice (Mounho & Thrall, 1999).

As a MAP kinase inhibitor, apigenin downregulated expression of the Na⁺/Ca²⁺ exchanger, a protein important for calcium extrusion, in neonatal rat cardiac myocytes (Carrillo *et al.*, 1998). By interfering with MAP kinase activity, apigenin was also able to significantly reduce epidermal growth factor (EGF)-induced SKBR-3 breast cancer cell proliferation and basement membrane invasion (Reddy *et al.*, 1999).

In the human anaplastic thyroid carcinoma cell line (ARO), apigenin treatment resulted in a decrease in the level of phosphorylated EGF receptor tyrosine kinase, as well as MAP kinases and their nuclear substrate, c-Myc, ultimately triggering apoptosis (Yin *et al.*, 1999).

Inhibition of Cell Cycle Progression. Apigenin was shown to induce morphological differentiation and G₂/M-phase cell cycle arrest in B104 rat neuronal cells (Sato *et al.*, 1994). Similarly, it induced a dramatic accumulation of G₂/M cells in rat hepatoma 5L and BP8 cells (Reiners *et al.*, 1999). Apigenin induced reversible cell cycle arrest in G₂/M phase in C50 and 308 mouse keratinocytes and in human HL-60 cells, which occurred via inhibition of p34^{cdc2}H1 kinase activity related to induction of cyclin B1 (Lepley *et al.*, 1996).

Treatment of asynchronous human diploid fibroblasts with 10-50 µM apigenin produced both the G₂/M and G₀/G₁ cell cycle arrest. The latter appeared to be mediated by the apigenin-induced inhibition of the Cdk2 kinase activity and the phosphorylation of retinoblastoma (Rb) protein, as well as via the induction of the Cdk inhibitor p21/WAF1, a downstream effector of the p53 tumor suppressor protein (Lepley & Pelling, 1997).

Induction of Apoptosis. Apigenin was shown to induce a dose-dependent reduction in viability of human promyelocytic leukemia HL-60 cells (IC₅₀ = 50 µM) through a rapid induction of caspase-3 activity, proteolytic cleavage of poly-(ADP-ribose) polymerase (PARP), loss of mitochondrial transmembrane potential, elevation of reactive oxygen species (ROS) production, release of mitochondrial cytochrome *c* into the cytosol, and subsequent induction of procaspase-9 processing (Wang *et al.*, 1999).

Effects on DNA Metabolism. Apigenin inhibited eukaryotic topoisomerase I (Topo I)-catalyzed DNA religation by forming a ternary complex with Topo I and the DNA substrate during the cleavage reaction and thus preventing a subsequent religation reaction (Boege *et al.*, 1996).

Antioxidant and Pro-oxidant Effects. Flavonoids such as apigenin have been identified as major cancer-preventive components of human diets due in part to their anti-oxidative, free radical scavenging, and anti-inflammatory activities (Galati *et al.*, 1999). Apigenin significantly induced glutathione transferase (GST) in Wistar rat heart, suggesting that it might function as a specific protectant against cardiotoxic agents (Breinholt *et al.*, 1999). The increase in

heart GST may help explain the epidemiological data that link flavonoid intake with a decreased risk of cardiovascular disease (Breinholt *et al.*, 1999; Hertog *et al.*, 1993).

Pro-oxidant properties of apigenin were demonstrated by studies that showed highly efficient apigenin-induced oxidation of glutathione (GSH) and NADH in the presence of peroxidase and hydrogen peroxide. Further research was suggested to determine the consequences, if any, for activated oxygen species formation by apigenin in peroxidase-containing tissues such as bone marrow or thyroid (Chan *et al.*, 1999; Galati *et al.*, 1999).

Antimutagenic Activities. In the *S. typhimurium* assay, apigenin inhibited benzo[*a*]pyrene (BaP)- and 2-aminoanthracene (2-AA)-induced bacterial mutagenesis. However, mutagenesis induced by methylnitrosourea (MNU) and methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was not inhibited by apigenin (Birt *et al.*, 1986).

Reproductive Effects. Apigenin showed dose-dependent and significant anti-implantation activity when administered to rats from days 1-4 of pregnancy. In immature ovariectomized rats, oral administration of apigenin caused a significant increase in uterine weight and diameter, as well as thickness of the endometrium and its epithelial height, thus exhibiting estrogenic properties (Hiremath *et al.*, 2000).

Structure-Activity Analysis: Flavonoids are a large group of polyphenolic compounds that comprise an important class of secondary metabolites in plants. Their chemical structure is based on the phenylchromane or flavane ring system (Paladini *et al.*, 1999). The most important groups of flavonoids are anthocyanins, flavonols, flavones, catechins, and flavanones. Apigenin belongs to the flavone group of flavonoids (Hertog *et al.*, 1993).

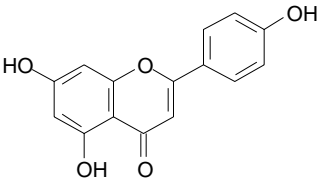
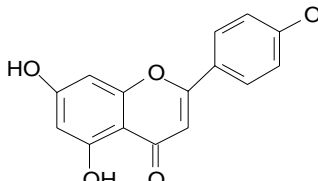
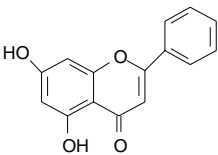
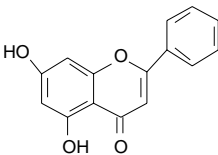
For this project, four flavonoids (acacetin, chrysin, luteolin, and norwogonin) were selected based on 90 percent or greater structural similarity to apigenin according to the National Library of Medicine (NLM) database, ChemIDplus

(NLM, 2000a). The *Chemical Carcinogenesis Research Information System* (CCRIS) and the *Registry of Toxic Effects of Chemical Substances* were searched for information on mutagenicity and carcinogenicity (Dialog, 2000; NLM, 2000b). Other citations from NLM's databases were identified on a case-by-case basis.

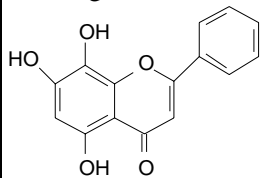
No 2-year carcinogenicity studies of the selected chemicals were identified in the available literature.

Table 1 provides a summary of information found on the genotoxicity of the selected compounds. Czczot and coworkers felt that the mutagenicity of flavonoids is dependent on the presence of hydroxyl groups in the 3' and 4' positions of the B ring, and that the presence of a free hydroxy or methoxy group in the 7 position of the A ring also probably contributes to the appearance of mutagenic activity in the Ames test. The presence of methoxy groups, particularly in the B ring markedly decreases mutagenic activity (Czczot *et al.*, 1990).

Table 1. Information on genotoxicity of selected naphthoquinones

Chemical Name	Mutagenicity Data
<p>Apigenin [520-36-5]</p> 	<p><i>S. typhimurium</i> TA97, TA98, TA100, TA102, TA1535, TA1538 with/without S-9: Negative for reverse mutations (Czeczot <i>et al.</i>, 1990; NLM, 2000b)</p> <p><i>E. coli</i> K-12 strain PQ 37 with/without S-9 metabolic activation: Weakly induced SOS repair system (Czeczot & Bilbin, 1991)</p> <p>Inhibits topoisomerase I-catalyzed DNA religation (Boege <i>et al.</i>, 1996)</p> <p><i>S. typhimurium</i> TA98, TA100. Weakly mutagenic (apigenin triacetate) (Nagao <i>et al.</i>, 1981)</p>
<p>Acacetin [480-44-4]</p> 	<p><i>S. typhimurium</i> TA98, TA100. Weakly mutagenic (Nagao <i>et al.</i>, 1981)</p> <p>Inhibits topoisomerase I-catalyzed DNA religation (Boege <i>et al.</i>, 1996)</p>
<p>Chrysin [480-40-0]</p> 	<p><i>S. typhimurium</i> TA98, not mutagenic in histidine reversion system (Hardigree & Epler, 1978)</p> <p>Human fibroblasts, RNA synthesis, and mouse RNA polymerase II transcription, weak inhibition (Nose, 1984)</p>
<p>Luteolin [491-70-3]</p> 	<p><i>S. typhimurium</i> TA1538, did not introduce damage into DNA recognized by UvrABC nuclease (Czeczot & Kustelak, 1993)</p> <p><i>S. typhimurium</i> TA97, TA98, TA100, TA102, TA1535, TA1538 with/without S-9: Negative for reverse mutations (Czeczot <i>et al.</i>, 1990)</p> <p><i>E. coli</i> K-12 strains PQ37 and PQ243, weakly induced SOS system (Czeczot & Kusztelek, 1993)</p> <p>Human lymphocytes, micronuclei & SCE at 10 mg/L (Dialog, 2000; Popp & Schimmer, 1991)</p>

Norwogonin [4443-09-0]



S. typhimurium. Strong mutagen in TA100 with S-9 but much weaker response in TA98 (Elliger *et al.*, 1984)

References

- A.G. Scientific Inc. (2000) Apigenin, A-1108. Cell Cycle Products. [http://www.agscientific.com/cellcycle.htm]
- Birt, D.F., Mitchell, D., Gold, B., Pour, P. & Pinch, H.C. (1997) Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. *Cancer Res.*, **17**, 85-92
- Birt, D.F., Walker, B., Tibbel, M.G. & Bresnick, E. (1986) Anti-mutagenesis and anti-promotion by apigenin, robinetin, and indole-3-carbinol. *Carcinogenesis*, **7**(6), 959-963 [abstract]
- Block, M.J. (2000) *Chemycyclopedia 2001*, vol. 19, Washington, DC, American Chemical Society, p 107
- Boege, F., Straub, T., Kehr, A., Boesenberg, C., Christiansen, K., Anderson, A., Jakob, F. & Kohrle, J. (1996) Selected novel flavones inhibit the DNA binding or the DNA religation step of eukaryotic topoisomerase I. *J. Biol. Chem.*, **271**(4), 2262-2270
- Breinholt, V., Lauridsen, S.T. & Dragsted, L.O. (1999) Differential effects of dietary flavonoids on drug metabolizing and antioxidant enzymes in female rat. *Xenobiotica*, **29**(12), 1227-1240
- Budavari, S., ed. (1997) *The Merck Index*, 13th ed. Whitehouse Station, NJ, Merck & Co., Inc., pp. 123-124
- Carrillo, C., Cafferata, E.G., Genovese, J., O'Reilly, M., Roberts, A.B. & Santa-Coloma, T.A. (1998) TGF-1 up-regulates the mRNA for the Na⁺/Ca²⁺ exchanger in neonatal rat myocytes. *Cell Mol. Biol.*, **44**(3), 543-551 [abstract]
- Chan, T., Galati, G. & O'Brien, P.J. (1999) Oxygen activation during peroxidase catalyzed metabolism of flavones or flavanones. *Chem. Biol. Interact.*, **122**(1), 15-25
- Chaumontet, C., Droumaguet, C., Bex, V., Heberden, C., Gaillard-Sanchez, I. & Martel, P. (1997) Flavonoids (apigenin, tangeretin) counteract tumor promoter-induced inhibition of intercellular communication of rat liver epithelial cells. *Cancer Lett.*, **114**(1-2), 207-210
- Chemical Sources International (2000) All chemical suppliers for apigenin. *Chem Sources Chemical Search* [http://kw1.innova.net]
- Czeczot, H. & Bilbin, M. (1991) Effect of flavones and their metabolites on induction of SOS repair in the strain PQ37-*E. coli* K-12. *Acta Biochim. Pol.*, **38**(1), 71-74

Czeczot, H. & Kusztelek, J. (1993) A study of the genotoxic potential of flavonoids using short-term bacterial assays. *Acta Biochim. Pol.*, **40**(4), 549-554 [abstract]

Czeczot, H., Tudek, B., Kusztelek, J., Szymczyk, T., Dobrowolska, B., Glinkowska, G., Malinowski, J. & Strzelecka, H. (1990) Isolation and studies of the mutagenic activity in the Ames test of flavonoids naturally occurring in medical herbs. *Mutat. Res.*, **240**(3), 209-216 [abstract]

Dialog Information Services (2000) RTECS (*Registry of Toxic Effects of Chemical Substances*) (File 336), Palo Alto, CA, searched May 2000 [Accession Nos. 031616, 060037, 060107, 060116, 060117]

Duke, J.A. & Beckstrom-Sternberg, S.M. (2000) Apigenin. *Dr. Duke's Phytochemical and Ethnobotanical Databases*. [<http://www.ars-grin.gov/cgi-bin/duke>]

Eaton, E.A., Walle, U.K., Lewis, A.J., Hudson, T., Wilson, A.A. & Walle, T. (1996) Flavonoids, potent inhibitors of the human P-form phenolsulfotransferase. Potential role in drug metabolism and chemoprevention. *Drug Metab. Dispos.*, **24**(2), 232-237

Elliger, C.A., Henika, P.R. & MacGregor, J.T. (1984) Mutagenicity of flavones, chromones and acetophenones in *Salmonella typhimurium*. New structure-activity relationships. *Mutat. Res.*, **135**(2), 77-86 [abstract]

FDA (1995) Food and Drug Administration, *Dietary Supplement Health and Education Act of 1994*. [<http://vm.cfsan.fda.gov/~dms.dietsupp.html>]

Fisher Scientific (2000) Apigenin. *Catalogs*. [<http://www3.fishersci.com>]

Galati, G., Chan, T., Wu, B. & O'Brien, P.J. (1999) Glutathione-dependent generation of reactive oxygen species by the peroxidase-catalyzed redox cycling of flavonoids. *Chem. Res. Toxicol.*, **12**(6), 521-525

Galijatovic, A., Otake, Y., Walle, U.K. & Walle, T. (1999) Extensive metabolism of the flavonoid chrysin by human Caco-2 and Hep G2 cells. *Xenobiotica*, **29**(12), 1241-1256

Griffiths, L.A. & Smith, G.E. (1972) Metabolism of apigenin and related compounds in the rat. *Biochem. J.*, **128**, 901-911

Gruenwald, J., Brendler, T. & Jaenicke, C., eds. (1998) *Matricaria Chamomilla* (Chamomile). *PDR for Herbal Medicines*. Montvale, NJ., Medical Economics Company, Inc., pp 961-962

Hardigree, A.A. & Epler, J.L. (1978) Comparative mutagenesis of plant flavonoids in microbial systems. *Mutat. Res.*, 58(2-3), 231-239 [abstract]

Healthnotes (1999) *Chamomile (Matricaria recutita)* [<http://www.puritanspride.com/ealthnotes>]

Hertog, M.G.L., Fesens, E.J.M., Hollman, P.C.H., Katan, M.B. & Kromhout, D. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet*, **342**, 1007-1011

Hiremath, S.P., Badami, S., Hunasagatta, S.K. & Patil, S.B. (2000) Antifertility and hormonal properties of flavones of *Striga orobanchioides*. *Eur. J. Pharmacol.*, **391**(1-2), 193-197 [abstract]

Hoffman, D.L. (2000a) Passion flower. *Herbal Materia Medica*. [<http://www.npicenter.com/library/herbal/materiamedica/PASSION.asp>]

Hoffman, D.L. (2000b) Chamomile. *Herbal Materia Medica*. [<http://www.npicenter.com/library/herbal/materiamedica/CHAMOMIL.asp>]

Janssen, K., Mensink, R.P., Cox, F.J., Harryvan, J.L., Hovenier, R., Hollman, P.C.F., Katan, M.B. (1998) Effects of the flavonoids quercetin and apigenin on hemostasis in healthy volunteers: results from an *in vitro* and a dietary supplement study. *Am. J. Clin. Nutr.*, **67**(2), 255-262 [abstract]

Jeong, H.J., Shin, Y.G., Kim, I.H. & Pezzuto, J.M. (1999) Inhibition of aromatase activity by flavonoids. *Arch. Pharm. Res.*, **22**(3), 309-312 [abstract]

Le Bail, J.C., Laroche, T., Marre-Fournier, F. & Habrioux, G. (1998) Aromatase and 17 -hydroxysteroid dehydrogenase inhibition by flavonoids. *Cancer Lett.*, **133**, 101-106

Lee, S.F. & Lin, J.-K. (1997) Inhibitory effects of phytopolyphenols on TPA-induced transformation, PKC activation, and *c-jun* expression in mouse fibroblast cells. *Nutr. Cancer*, **28**(2), 177-183

Lepley, D.M., Li, B., Birt, D.F. & Pelling, J.C. (1996) The chemopreventive flavonoid apigenin induces G₂/M arrest in keratinocytes. *Carcinogenesis*, **17**(11), 2367-2375

Lepley, D.M. & Pelling, J.C. (1997) Induction of p21/WAF1 and G₁ cell-cycle arrest by the chemopreventive agent apigenin. *Mol. Carcinog.*, **19**(2), 74-82

Li, B., Pinch, H. & Birt, D.F. (1996) Influence of vehicle, distant topical delivery, and biotransformation on the chemopreventive activity of apigenin, a plant flavonoid, in mouse skin. *Pharm. Res.*, **13**(10), 1530-1534

Lide, D.R., ed. (1997) *CRC Handbook of Chemistry and Physics*, 78th ed., New York, CRC Press, pp 3-78

Lin, J.-K., Chen, Y.C., Huang, Y.-T., & Lin-Shiau, S.Y. (1997) Suppression of protein kinase C and nuclear oncogene expression as possible molecular mechanisms of cancer chemoprevention by apigenin and curcumin. *J. Cell. Biochem. Suppl.*, **28-29**, 39-48

Merfort, I., Heilmann, J., Hagedorn-Leweke, U. & Lippold, B.C. (1994) *In vivo* skin penetration studies of camomile flavones. *Pharmazie*, **49**(7), 509-511

Mounho, B.J. & Thrall, B.D. (1999) The extracellular signal-regulated kinase pathway contributes to mitogenic and anti-apoptotic effects of peroxisome proliferators *in vitro*. *Toxicol. Appl. Pharmacol.*, **159**(2), 125-133

Nagao, M., Morita, N., Yahagi, T., Simizu, M., Kuroyanagi, M., Fukuoka, M., Yoshihira, K., Natori, S., Fujino, T. & Sugimura, T. (1981) Mutagenicities of 61 flavonoids and 11 related compounds. *Environ. Mutagen.*, **3**(4) 401-419 [abstract]

Nemecz, G. (2000) *Herbal Pharmacy: Chamomile*. [http://152.38.18.3/~nemecz/George_home/references/Chamomile.html]

Nielsen, S.E., Breinholt, V., Justesen, U., Cornett, C. & Dragsted, L.O. (1998) *In vitro* biotransformation of flavonoids by rat liver microsomes. *Xenobiotica*, **28**(4), 389-401

Nielsen, S.E., Young, J.F., Daneshvar, B., Lauridsen, S.T., Knuthsen, P., Sandström, B. & Dragsted, L.O. (1999) Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion, blood antioxidant enzymes, and biomarkers for oxidative stress in human subjects. *Br. J. Nutr.*, **81**, 447-455

NLM (1999) RTECS (*Registry of Toxic Effects of Chemical Substances*), Bethesda, MD, National Library of Medicine, searched May 2000 [Record No. 58596]

NLM (2000a) *ChemIdplus*, National Library of Medicine, Bethesda, MD, searched May 2000 [Record Nos. 480-40-0, 480-44-4, 491-67-8, 491-70-3, 491-71-4, 520-36-5, 4443-09-8].

NLM (2000b) *CCRIS (Chemical Carcinogenesis Research Information System)*, National Library of Medicine, Bethesda, MD, searched May and November 2000 [Record Nos. 3789, 3790]

Nose, K. (1984) Inhibition by flavonoids of RNA synthesis in permeable WI-38 cells and of transcription by RNA polymerase II. *Biochem. Pharmacol.*, **33**(23), 3823 [abstract]

Paladini, A.C., Marder, M., Viola, H., Wolfman, C., Wasowski, C. & Medina, J.H. (1999) Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. *J. Pharm. Pharmacol.*, **51**(5), 519-526

Pforte, H., Hempel, J. & Jacobasch, G. (1999) Distribution pattern of flavonoid extract in the gastrointestinal lumen and wall of rats. *Nahrung*, **43**(3), 205-208

PHS-149 (1972) *Survey of Compounds which Have Been Tested for Carcinogenic Activity*, National Cancer Institute, U.S. Department of Health and Human Services, p1042

Popp, R. & Schimmer, O. (1991) Induction of sister-chromatid exchanges (SCE), polyploidy, and micronuclei by plant flavonoids in human lymphocyte cultures. A comparative study of 19 flavonoids. *Mutat. Res.*, **246**(1), 205-213 [abstract]

Redaelli, C. (1982) Extractive process for preparing apigenin. Assignee: Benomelli S.p.A., (US Patent 4,313,880), 4 pp

Reddy, K.B., Krueger, J.S., Kondapaka, S.B. & Diglio, C.A. (1999) Mitogen-activated protein kinase (MAPK) regulates the expression of progelatinase B (MMP-9) in breast epithelial cells. *Int. J. Cancer*, **82**(2), 268-273

Reiners, J.J., Jr., Clift, R. & Mathieu, P. (1999) Suppression of cell cycle progression by flavonoids: dependence on the aryl hydrocarbon receptor. *Carcinogenesis*, **20**(8), 1561-1566

Sato, F., Matsukawa, Y., Matsumoto, K., Nishino, H. & Sakai, T. (1994) Apigenin induces morphological differentiation and G2-M arrest in rat neuronal cells. *Biochem. Biophys. Res. Commun.*, **204**(2), 578-584

Sigma-Aldrich (2000) Apigenin (product numbers A3145, 10798, and 460745). *Product Search*. [<http://www.sigma-aldrich.com/sacatalog.nsf/productlookup>]

Tocris (2000) Cell cycle and apoptosis reagents. *On-line Catalogue*. [<http://www.tocris.com/cat/cellcycle.html>]

USPTO (2000) United States Patent and Trademark Office, Results of search for all years db for: apigenin: 107 patents. *Patent Full Text and Image Database*. [<http://www.uspto.gov/patft/index.html>]

Wang, I.-K., Lin-Shiau, S.-Y. & Lin, J.-K. (1999) Induction of apoptosis by apigenin and related flavonoids through cytochrome c release and activation of caspase-9 and caspase-3 in leukemia HL-60 cells. *Eur. J. Cancer*, **35**(10), 1517-1525

Webmd.com (2000) Chamomile flower, German. *Alternative Herbal Index*.
[<http://onhealth.webmd.com/alternative/resource/herbs>]

Wei, H., Tye, L., Bresnick, E. & Birt, D.F. (1990) Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. *Cancer Res.*, **50**(3), 499-502 [abstract]

Yin, F., Giuliano, A.E. & Van Herle, A.J. (1999) Signal pathways involved in apigenin inhibition of growth and induction of apoptosis of human anaplastic thyroid cancer cells (ARO). *Anticancer Res.*, **19**(5B), 4297-4304