β-Caryophyllene: A Sesquiterpene with Countless Biological Properties

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Abstract: *β*-Caryophyllene (BCP), a natural bicyclic sesquiterpene, is a selective phytocannabinoid agonist of type 2 receptors (CB2-R). It isn't psychogenic due to the absence of an affinity to cannabinoid receptor type 1 (CB1). Among the various biological activities, BCP exerts anti-inflammatory action via inhibiting the main inflammatory mediators, such as inducible nitric oxide synthase (iNOS), Interleukin 1 beta (IL-1β), Interleukin-6 (IL-6), tumor necrosis factor-alfa (TNF-α), nuclear factor kappa a-light-chain-enhancer of activated B cells (NF-κB), cyclooxygenase 1 (COX-1), cyclooxygenase 2 (COX-2). Peroxisome proliferator-activated receptors alpha (PPAR-α) effects are also mediated by the activation of PPAR-α and PPAR-γ receptors. In detail, many studies, in vitro and in vivo, suggest that the treatment with *β*-caryophyllene improves the phenotype of animals used to model various inflammatory pathologies, such as nervous system diseases (Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, stroke), atherosclerosis, and tumours (colon, breast, pancreas, lymphoma, melanoma and glioma cancer). Furthermore, pre-clinical data have highlighted that BCP is potentially useful in *Streptococcus* infections, osteoporosis, steatohepatitis, and exerts anticonvulsant, analgesic, myorelaxing, sedative, and antidepressive effects. BCP is non-toxic in rodents, with a Lethal dose, 50% (LD50) greater than 5000 mg/kg. Nevertheless, it inhibits various cytochrome P450 isoforms (above all, CYP3A4), which metabolise xenobiotics, leading to adverse effects, due to drug levels over therapeutic window. All the reported data have highlighted that both pharmacological and toxicological aspects need to be further investigated with clinical trials.

Keywords: sesquiterpene; inflammatory pathologies; nutraceuticals

1. Introduction

Sesquiterpenoids are an extremely wide group of secondary metabolites found in the plant kingdom [1,2]. Their main carbon backbone consists of 15 atoms and several synthases action leads to a great variety of chemical structures. In particular, bicyclic sesquiterpenoids have raised attention in the bio-pharmacological field, even as essential oils. Among them, *β*-caryophyllene is one of the major component of essential oils extracted from spice and food plants [3–7], such as black pepper (*Piper nigrum* L.), rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum* spp.)

oregano (*Origanum vulgare* L.) basil (*Ocimum* spp.), thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), mint *(Mentha piperita*), ginger (*Zingiber officinale*), chinotto (*Citrus Myrtfolia* Raf) [8], and cloves (*Syzygium aromaticum*) [9,10]. It was also found in citronella, (*Cymbopogon*), pine tree (*Pinus*), *Chenopodium ambrosioides*, *Cannabis sativa*, in plants of the genus *Copaifera*, *Artemisia*, *Murraya*, *Cordia*, *Spiranthes*, *Ocimum*, *Croton* [9,10], and in the leaves of *Annona Cherimola* [11]. This plant compound was approved by Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) and it is used as flavour enhancer [9] and in cosmetics [12]. In nature, BCP mainly occurs as *trans*-caryophyllene ((E)-BCP) (**1**) mixed with small amount of the isomers (Z)-*β*-caryophyllene ((Z)-BCP) (**2**) and *α*-humulene (*α*-caryophyllene) (**3**) [12], and its oxidation derivative, *β*-caryophyllene oxide (BCPO) (**4**) (Figure 1).
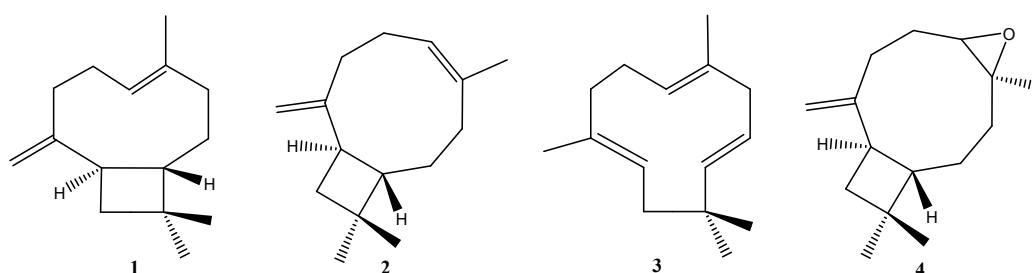

Figure 1. Structures of sesquiterpenes (**1–4**).

BCP belongs to cannabinoid family, which are ligands of the cannabinoid receptors present in the organism. Cannabinoid receptors CB1-R and CB2-R are metabotropic receptors are G protein (protein binding GTP)-coupled receptors, involved in the regulation of neurotransmitters responsible for maintaining an energetic balance, in the metabolism, and in the immune response. The aforementioned receptors are bound and activated by endogenous cannabinoids, derivatives of arachidonic acid, including 2-arachidonoylglycerol and *N*-arachidonoylethanolamine, better known as anandamide. Both receptors are bound by a lot of proteins in various pathways, acting as mediators of cellular responses to biological molecules.

Unlike the main traditional cannabinoids, such as Δ^8-tetrahydrocannabinol (**5**), Δ^9-tetrahydrocannabinol (**6**) and cannabinol (**7**) (Figure 2), able to activate both receptors, BCP has a very different chemical structure and it is a selective agonist of CB2-R. Furthermore, BCP has no side effects, not activating CB1-Rs (mainly expressed in the central nervous system, but also in the liver, lungs, heart, blood vessels and digestive tract). CB2-Rs are mainly found in peripheral tissues and in immune system cells (B and NK lymphocytes, macrophages, mast cells) and, to a lesser extent, in the central nervous system (brain, neurons, microglia) [13]. The expression of CB2-Rs in the central nervous system is increased in neurodegenerative pathologies, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and in some tumours like gliomas [14].

The binding of BCP to CB2-Rs is responsible for many cellular events:

- The activation of $G_{i/o}$ *α*-subunit inhibits the activity of adenylate cyclase, the enzyme which converts ATP in cAMP (cyclic AMP) and activates phospholipase C (PLC), determining calcium release from IP3 (inositol trisphosphate)-sensitive calcium channels, increasing the intracellular level of calcium;
- The activation of the complex formed by $G_{i/o}$ *β*- and *γ*-subunits, which modulate various pathways modifying the expression and the activity of many target proteins [15].
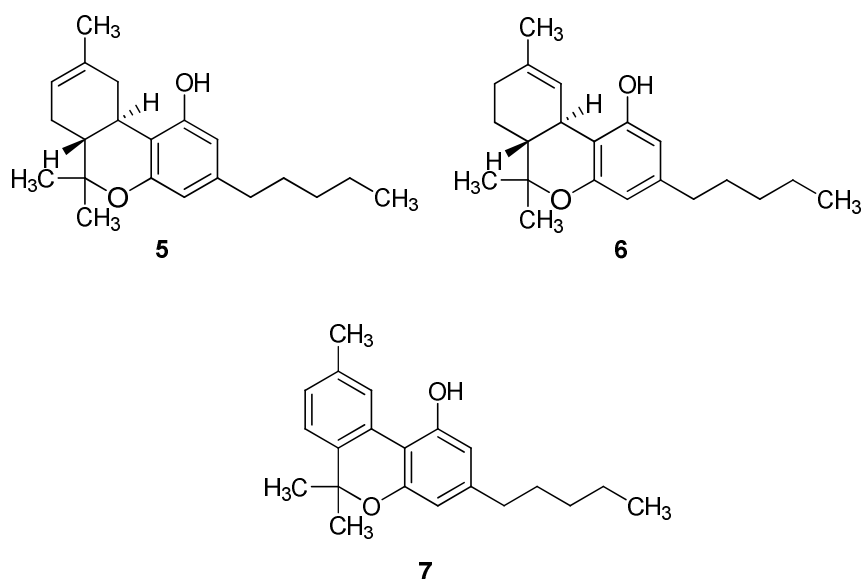
Figure 2. Structures of cannabinoids (**5**–**7**).

In addition to the involvement in physiological functions, CB2-Rs are implied in numerous pathological processes, so they can represent an interesting target in order to obtain agonist molecules for the treatment of many pathological conditions, including neuropathic pain, inflammation, neuroinflammatory and neurodegenerative pathologies (Parkinson's disease, Alzheimer's disease, multiple sclerosis, and amyotrophic lateral sclerosis), spinal and brain injuries, stroke, ischemia, anxiety disorder, depression, colitis, fibrosis and liver ischemia, atherosclerosis, osteoporosis, osteoarthritis, diabetes, obesity, and some types of cancer [9,12–14,16–21].

Molecular docking studies showed that BPC interacts with CB2-R on the binding site of CP55,940 (an agonist), precisely in a hydrophobic pocket, engaging with hydrophobic amino acid residues (valine-113, phenylalanine-117, isoleucine-198, tryptophan-258 and methionine-265) [12]. The double bond with conformation E of BCP is fundamental for receptor binding. BCPO lacks a double bond in position C4-C5 and it is not able to bind CB2-R [10].

2. Biological properties of *β*-caryophyllene

In the last few years, BCP has represented an important subject of study [9–15,22–65]. In particular, a lot of researches about the its effects in vitro and in vivo on animals have been conducted, thus experimental data about its biological properties have been verified. However, further studies are needed in order to translate the findings in animal models into promising pre-clinical and clinical trials on humans. Pre-clinical studies have revealed that BCP is a modulator of nervous system and exerts beneficial effects on numerous neurodegenerative and inflammatory pathologies. Moreover, it is able to act on the liver and bones, and has antibiotic properties.

Table 1 shows the principal studies on *β*-caryophyllene.

Table 1. Preclinical studies on *β*-caryophyllene.

Experimental Conditions	Effects	Possible Mechanisms	References
50 mg/kg (i.p.) in Wistar rats (models of Alzheimer's disease)	Neuroprotective	Decreased NO synthesis; Decreased activation of astrocytes and microglia; Reduced expression of Iba-1 e GFAP	Ojha et al. (2016) [22]
10 mg/kg in rat Clone 6 (C6) microglial cells	Anti-inflammatory	Reduced production of iNOS, IL-1β, IL-6 and COX-2	Chang et al. (2013) [24]
10, 25, and 50 μM in BV2 mouse cells	Suppression of neuroinflammation due to hypoxia	NF-κB inhibition; Decreased production of NO and Prostaglandin E2 (PGE2)	Hu et al. (2017) [26]
10 mg/kg in SH-SY5Y mouse cells treated with MPTP (model of Parkinson's disease)	Neuroprotective; enhancement of motor coordination	Nrf2 activation	Viveros-Paredes et al. (2017) [30]
0.5 and 1 μM in rat C6 glioma cells	Antioxidant	Nrf2 activation	Assis et al. (2014) [33]
25 mg/kg twice a day (p.o.) in mice treated with paclitaxel (model of peripheral neuropathy)	Attenuation of peripheral neuropathy	mitogen-activated protein kinase (MAPK) p38/NF-κB inhibition	Segat et al. (2017) [36]
2.25–18 μg (s.c.)	Strengthening of analgesic effect of morphine	CB2-R activation; Stimulation of β-endorphins release and μ-opioid receptors	Katsuyama et al. (2013) [37]
50 mg/kg twice a day in mice with experimental autoimmune encephalomyelitis (model of multiple sclerosis)	Decreased pain; decreased cerebral damage; antioxidant	Decreased production of IFN-γ; Increased expression of IL-10; Increased activity of catalase, superoxide dismutase and glutathione peroxidase.	Alberti et al. (2017) [38]
50, 100, and 150 mg/kg of the hydroalcoholic extract and 200 mg/kg of the essential oil from basil in Syrian mice.	Sedative		Rabbani et al. (2015) [39]
50, 100 mg/kg of the essential oil from *Baccharis uncinella* (p.o.)	Sedative		Ascari et al. (2012) [40]
50, 100, 150 mg/kg of the hydroalcoholic extract and 200 mg/kg of the essential oil from basil (i.p.) in Syrian mice	Anxiolytic		Rabbani et al. (2015) [39]
50 mg/kg in (C57 black 6) C57BL/6 mice (p.o.)	Anxiolytic	Activation of CB2-Rs	Bahi et al. (2014) [41]
100 and 300 μg/mL of essential oil from *Pterodon polygalaeflorus* and 30 and 100 μg/mL of BCP in rats	Muscle relaxant		Leonhardt et al. (2010) [42]
50 mg/kg in C57BL/6 mice (p.o.)	Antidepressive	Activation of CB2-Rs	Bahi et al. (2014) [41]

Table 1. *Cont.*

Experimental Conditions	Effects	Possible Mechanisms	References
10, 30 and 100 mg/kg in C57BL/6 mice	Anticonvulsant		de Oliveira et al. (2016) [43]
30 mg/kg every four hours in mice	Anticonvulsant		Tchekalarova et al. (2018) [44]
In HT29 and HTC116 (cell lines of colon cancer)	Antitumour		Dahham et al. (2015) [45]
In PANC-1 (cell line of pancreas cancer)	Antitumour		Dahham et al. (2015) [45]
2.5, 5 and 10 μmol/L in B16F10 melanoma cells in high-fat diet-induced obese C57BL/6N mice	Reduction of obesity-related cancer risk		Jung et al. (2015) [48]
Essential oil from *Pamburus missionis* (containing also phytolandaromadendrene oxide) in A431 and HaCaT cell lines (skin epidermoid cancer)	Antitumour	ROS production; Increased Bcl-2 Associated X protein (Bax) expression; Reduced B-cell lymphoma 2 (Bcl-2) expression; Cytochrome C release in cytoplasm; Apoptosis.	Pavithra et al. (2018) [49]
In BS-24-1 (mouse cell line of lymphoma) and MoFir (human lymphocytes B transformed through Epstein-Barr virus)	Antitumour		Pavithra et al. (2018) [49]
In MCF-7 (breast cancer), L-929 (mouse fibroblasts), DLD-1 (colon cancer)	Increased intracellular levels of paclitaxel	Increased permeability of cell membrane; CYP3A4 inhibition	Fidyt et al. (2016) [13]; Legault et al. (2007) [47]; Nguyen et al. (2017) [64]
In CT26 cells transplanted in BALB/c mice and exposed to high levels of glucose (model of colorectal cancer)	Antitumour	NF-κB and arginine ADP-ribosyltransferase 1 (ART1) inhibition	Zhou et al. (2018) [51]
1 or 10 μg/mL + baicalin (1, 10 μg/mL) and (+)-catechin (1, 10 μg/mL) in macrophages of RAW267.4 mouse	Reduction of macrophage proliferation	Cell cycle arrest G2/M phase; Reduction of the expression of Akt, MAPK e p65 NF-κB; Caspase-3 activation	Yamaguchi et al. (2016) [52]
30 mg/kg in high-fat-fed Wistar rats (p.o.)	Antiatherogenic; Antioxidant	Activation of cannabinoid receptor 2 (CB2-R), PPAR-γ, PPAR-α, PGC1-α; Reduction of Vascular cell adhesion molecule-1 (VCAM1) expression	Youssef et al. (2019) [53]
40 mg in Wistar rats (model of bilateral carotid artery occlusion and reperfusion)	Prevention of the effects induced bycarotid occlusion and reperfusion	Activation of endocannabinoid system; Increased PPAR-α expression	Poddighe et al. (2018) [55]

Table 1. *Cont.*

Experimental Conditions	Effects	Possible Mechanisms	References
215 and 430 mg/kg/die (p.o.) in rats (model of rheumatoid arthritis)	Reduction of oxidative stress, inflammation and edema	Radical scavenging; Nrf2 induction; Increased glutathione levels; Reduction of the expression of COX-2, cytokines and NF-κB	Ames-Sibin et al. (2018) [56]
30 and 300 mg/kg (p.o.) in C57BL/6J mice fed with methionine- and choline-deficient diet (model of non-alcoholic steatohepatitis, NASH)	Reduction of liver steatosis, inflammation and fibrosis	Reduction of cytokineslevels Transforming growth factor beta (TGF-β), Nox2 and collagen; Increased levels of SOD2 and GPx1	Arizuka et al. (2017) [58]
In the oral cavity for about 10 min	Bactericidal against Streptococcus mutans (tooth decay)	GlucosyltransferaseB (GtfB), GtfC and GtfD Inhibition	Yoo et al. (2018) [59]
0.1–100 μM in culture of mouse bone marrow cells	Stimulates osteoblast mineralization and inhibits adipogenesis and osteoclastogenesis	PPAR-γ activation in pluripotent stem cells; Inhibits TNF-α and NF-κB in osteoclasts	Yamaguchi et al. (2016) [61]

2.1. β-Caryophyllene and Nervous System

Many studies report the beneficial effects of BCP on central nervous system [22–44], in particular against neuroinflammatory and neurodegenerative pathologies.

Neuroinflammation is a process leading to nervous system degeneration, characterised by the activation of monocytes, macrophages, mast cells, lymphocytes, and the production of inflammation mediators, such as nitric oxide (NO), various cytokines (IL-1β, IL-6 and TNF-α), the protein NF-κB (nuclear factor kappa B) and the prostaglandins.

In detail, BCP, administered intraperitoneally in Wistar rats, at the dose of 50 mg/kg, has reduced the activity of inducible nitric oxide synthase (iNOS) and, consequently, nitric oxide production, thus decreasing brain oxidative stress and leading to the inhibition of lipid peroxidation and the depletion of glutathione stores [22], causing striatal and cortical neurotoxicity [9]. Moreover, nitric oxide is involved in the activation of cyclooxygenase (COX), the enzyme synthetizing prostaglandin H2 (PGH2), which is the precursor of the other prostaglandins, in particular Prostaglandin D2 (PGD2) and Prostaglandin E2 (PGE2), responsible for inflammation and pain. In this contest a frontiers of research for BCP and BCPO will be their influence on microRNA, small molecule able to regulate gene expression, considering that they are involved and proposed as biomarkers, in neuroinflammation and pain [66,67].

The essential oil of *Erymanthos erythropappus*, rich in BCP, has been shown to have anti-inflammatory in Wistar rats [23].

It has also been demonstrated that BCP, at the dose of 10 mg/kg, inhibits the transcription of iNOS, IL-1β, IL-6, and COX-2 in C6 microglia cells [9,24].

Furthermore, BCP, tested on the mouse BV2 cell line at the concentrations of 10, 25 and 50 μM, has inhibited NF-κB activation and reduced the production of nitric oxide and PGE2, thus suppressing hypoxia-induced neuroinflammatory response [25,26].

In the case of central nervous system pathologies, Iba-1, analogous protein of Aif-1, present only in macrophages and microglia, is over expressed (for example, in ischemia) [27], whereas GFAP (glial fibrillary acidic protein), which forms intermediate filaments, is expressed in a lot of central nervous system cells (including astrocytes and ependymal cells). In addition, it is involved in cell communication, in the interaction neuron-astrocytes, in the functioning of the blood–brain barrier

(BBB), particularly during mitosis, in which it modulates the filament network, and in the repair following brain injuries [9,28]. It has been demonstrated that BCP administered intraperitoneally at the dosage of 50 mg/kg, reduces the activation of astrocytes and microglia, by decreasing Iba-1 and GFAP expression, thus avoiding the death of dopaminergic neurons [22].

Amyloid plaques, formed by *β*-amyloid peptides, composed in turn by 36–43 amino acids and derived from amyloid precursor protein (APP), characterize Alzheimer's disease. These peptides are responsible for direct toxicity (death of neurons) and indirect toxicity (production of molecules stimulating inflammation). A considerable decrease of the *β*-amyloid peptide-induced overexpression of TLR4 (Toll-like receptor 4), which determines the activation of monocytes and microglia, has been noted in BV2 microglia cells treated with BCP, and, consequently, the sesquiterpene leads to a clear reduction of the biosynthesis of IL-6, IL-1β, PGE2, TNF-α, NF-κB and nitric oxide. In addition, also a reduced expression of COX-2 and iNOS has been observed [26].

Another common condition affecting central nervous system is Parkinson's disease, a neurodegenerative pathology characterized by neuroinflammation, oxidative stress, mitochondrial dysfunction and cell death, particularly in dopaminergic neurons. An in vitro study on SH-SY5Y cells showed that the treatment with BCP inhibits reactive oxygen species (ROS) production, restoring mitochondrial functionality and the levels of the antioxidant glutathione. BCP prevents apoptosis, by inhibiting the expression of Bax and caspase-3 and increasing Bcl-2 one. It reduces the phosphorylation of JNK (c-Jun N-terminal Kinase), which determines the increase of HO-1 (heme oxygenase-1) expression in this pathological condition. All these effects are related to CB2/Nrf2 pathway [29]. Research on a mouse model of Parkinson's disease conducted by Viveros-Paredes revealed that the treatment with BCP, at the dose of 10 mg/kg, enhances motor coordination in mice and protects the dopaminergic neurons from degeneration, reducing the production of inflammatory cytokines, in particular IL-1β, IL-6, and TNF-α [30].

BCP is also cytoprotective towards the central nervous system due to its modulation of the redox state and inflammation, useful during chemotherapy. The main mechanism of action regarding this aspect is the stimulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) following the activation of cannabinoid receptors CB2. Nrf2 is a transcription factor, whose activity is stimulated by oxidative stress (in particular, reactive oxygen species or ROS) and oncogenes, such as KRAS, BRAF and MYC. Nrf2 increases the expression of the genes involved in cell survival and in the reduction of the inflammatory process and the oxidative stress in SNC. Moreover, nerve growth factor (NGF) is stimulated through Tropomyosin receptor kinase A (Trk A) pathway, which stimulates in turn PI3K-Akt pathway [68].

BCP, through Nrf2 activation, increases the expression of antiapoptotic genes (B-cell leukemia/lymphoma-2 (BCL-2), Mouse double minute 2 (mdm-2), COX-2, c-Myb) and, at the same time, reduces the one of proapoptotic genes (Bax, Bak-1, caspase-8, caspase-9) [31].

All these cellular events determine cell survival and proliferation and angiogenesis [9].

BCP also diminishes the expression of matrix metallopeptidase (MMP-9) and increases the one of occludin, claudin-5, Tight Junction Protein ZO-1 and Growth Associated Protein 43 (GAP-43) [32].

Furthermore, BCP, at the concentrations of 0.5 and 1 μM, exerts cytoprotective effect on C6 glioma cells, increasing the antioxidant activity, through CB2-R-dependent Nrf2 pathway [33]. In addition, administered orally in Wistar rats, at the dosages of 34, 102 and 306 mg/kg, it reduces oxidative stress and neuronal apoptosis, due to the increased expression of Nrf2 and OH-1.

BCP also inhibits the production of nitric oxide, hydrogen peroxide, TNF-α, interferon gamma (IFN-γ), interleukin-17 (IL-17) reducing the extent of macrophage infiltration [9].

BCP can induce neurogenesis with a mechanism independent from the activation of CB2-R, Nrf2 and NGF, stimulating Tropomyosin receptor kinase A (TrkA) in SH-SY5Y (Cell lines Homo sapiens, human, bone marrow) and PC12 (Cell line derived from a pheochromocytoma of the rat adrenal medulla) neuroblastoma cells [34].

There are numerous studies regarding the analgesic effects of the sesquiterpene, isolated or in mixture in essential oils. In fact, the essential oil of *Erymanthos erythropappus*, containing BCP as one of the main components, administered orally at the doses of 200 and 400 mg/kg, induces analgesia in Wistar rats [23]. Further, the essential oil of *Senecio rufinervis*, containing about the 6% of BCP, is responsible for the analgesic effect in mice, when administered orally at the doses of 50 and 75 mg/kg [35].

Other studies have reported the same results in mice at the doses of 1 and 5 mg/kg intraperitoneally and at the dosages of 2.6 mg/kg/die per *os* for 2 weeks, alone or in combination with the docosahexaenoic acid (DHA) [9]. The essential oil of *Vitex agnus-castus* (BCP accounts for about 7% of the oil), has exhibited analgesic effect in Wistar rats which were subjected to an immersion test.

BCP, at the dose of 25 mg/kg, decreases the extent of peripheral neuropathyin mice, by the activation of cannabinoid receptors CB2-R and the inhibition of the MAPK p38, resulting in a reduced transcriptional activity of NF-κB, involved in phlogosis [36].

It has been demonstrated in mice that BCP actually exerts its antinociceptive action by activating CB2-Rs, in particular towards primary sensory neurons. In fact, the analgesic effect manifests itself in wild-type mice, but not in the CB2-R knockout mice. The activation of cannabinoid system indirectly leads to the modulation of opioid system. In more detail, it stimulates β-endorphin release, activating opioid receptors μ on primary afferent neurons. BCP has been shown to boost the painkilling effect of morphine, used in the treatment of severe pain, making possible the reduction of the dose of the drug and, consequently, decreasing its side effects [37].

Instead, there was no indication of synergy between BCP and the other components of the essential oils [13].

The derivative BCPO has not elicited any interest as an analgesic [13], since it does not bind to cannabinoid receptors due to the different molecular structure.

BCP could result potentially useful in multiple sclerosis management (pathology characterized by axon demyelination and neuroinflammation), as demonstrated by previous study which investigated the therapeutic potential of BCP on experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS).

The research has shown that the treatment with BCP reduces interferon-γ (IFN-γ) production, the main activator of macrophages, and increases the levels of interleukin-10 (IL-10).

Moreover, it inhibits cell T migration. The oral treatment with 50 mg/kg of BCP twice a day has decreased hyperalgesia induced by the EAE and protected from brain damage, inhibiting cytokine biosynthesis and restoring the activity of catalase, superoxide dismutase and glutathione peroxidase, all enzymes involved in the detoxification of oxidant substances [38].

Two essential oils containing BCP, basil one, administered intraperitoneally (at the doses of 100, 150 and 200 mg/kg), and *Baccharis uncinella* one, administered per *os* (at the doses of 50 or 100 mg/kg), exhibit significant sedative effect [39,40].

These early results on the animal model could be attributed to BCP, being a valid alternative to the most common sedatives and anaesthetics.

The latter drugs have contraindications in patients with cardiovascular, neuromuscular and cerebrovascular diseases, and could cause many side effects, such as convulsions, insomnia, agitation, dependence and, in the most extreme cases, lead to death.

Basil essential oil has also exerted anxiolytic effect [39]. The same results have been obtained in mice, when BCP has been orally administered at the dose of 50 mg/kg [41].

Therefore, all these data suggest the possible use of the sesquiterpene as a substitute of the most common anxiolytics, like benzodiazepines and the selective serotonin reuptake inhibitors (SSRI), which cause many adverse effects, such as movement problems, sedation, dependence, muscle relaxation, anterograde amnesia, teratogenic risk, reduction of bone mineral density.

BCP also exhibits antidepressant properties, as revealed by a research conducted on albino Swiss mice receiving 50 mg/kg of the BCP per Os (oral somministration). The mechanism of this effect is due to the activation of CB2-R [41].

Various studies have shown muscle relaxant effect of BCP in animals. In this regard, the compound, alone or as a constituent of the essential oil from *Pterodon polygalaeflorus*, exerts antispasmodic activity on rat isolated ileum at variable concentrations (1 to 1000 µg/mL) [42].

BCP, orally administered at the dosages of 10, 30, and 100 mg/kg in C57BL/6 mice, exerted a dose-dependent anticonvulsant effect [9,43]. Furher research on mice has revealed that the sesquiterpene inhibits tonic-clonic seizures in MES (maximal electro shock seizure) test and reduces kainate-induced neurotoxicity, suggesting a possible use of the sesquiterpene for the treatment of epilepsy [44].

2.2. β-Caryophyllene and Cancer

Both BCP and BCPO have shown cytotoxic activity against various cancer cell lines.

In particular, BCP has significantly decreased the proliferation of two colon cancer cell lines, HT-29 and HCT-116, and a pancreas cancer cell line, PANC-1. Moreover, it has been quite successful on other types of cancer cells [45].

In the intestinal cancer cell line CaCo-2 [46], for example, BCP has not been able to exert a significant effect on cell growth, unlike the isomer α-humulene [13].

In human breast cancer cells MCF-7, BCP amplifies the cytotoxicity of the isomers isocaryophyllene and α-humulene [47]. A study realized on obese mice C57BL/6N, injected with melanoma cells, has shown that the phytocannabinoid is able to decrease the precancerous effect caused by a high-fat diet [48].

BCP, which is more than 25% of the essential oil from *Pamburus missionis*, has synergistic effect with two other important components of the essential oil, phytol (**8**) and aromadendrene oxide (**9**) (Figure 3), resulting in antitumor activity against A431 and HaCaT cell lines, by blocking the cell in phase G0/G1 or sub-G1.
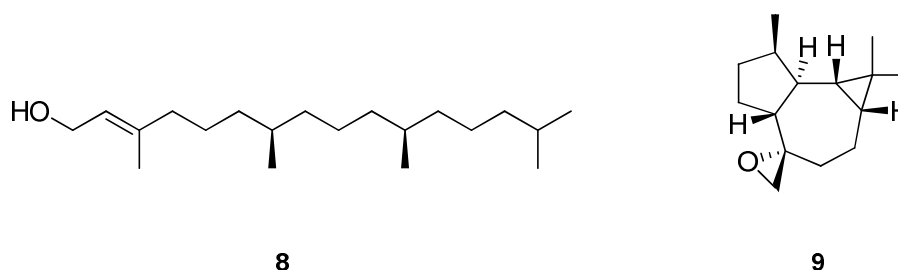

8   **9**

Figure 3. Structures of the phytol (**8**) and aromadendrene oxide (**9**).

The mechanism of action is associated to ROS production [69] and the mitochondrial membrane potential loss, by increasing Bax expression and decreasing Bcl-2 expression. Bax and Bak oligomers form pores, which increase the permeability of the external mitochondrial membrane, releasing cytochrome c in the cytoplasm, which is an apoptosis characterizing event [70–72]. The release of cytochrome c from mitochondria to cytosol leads to the formation of apoptosomes, and consequently, the activation of caspase-9, which activates the cascade of the effector caspases [49,73,74].

It has been shown that BCP causes the activation of caspase-3 and determines nucleolus fragmentation and the consequent apoptosis in two different cell lines, BS-24-1 (murine cell line of lymphoma) and MoFir (human T cell transformed through Epstein-Barr virus) [49].

BCP, one of the compounds in the essential oil, of *Commiphora gileadensis*, is responsible for antiproliferative exhibited by 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay [75–80] and proapoptotic effects (exhibited via DNA "ladder" and caspase-3 activation) in tumor cell lines, while there was no apoptosis induction in normal cell lines (FB) [13,50].

Anyhow, numerous studies have revealed that BCP enhances the effectiveness of antitumor drugs. In particular, a research has shown that it increases the activity of paclitaxel in lots of cell lines: MCF-7 (breast cancer), L-929 (mouse fibroblasts), DLD-1 (colon cancer) [47]. In detail, in the latter cell line, the BCP determines a rise of the intracellular concentrations in the drug, probably by increasing the permeability of the cell membrane [13].

BCP has been reported to exert anticancer and hypoglycemic effects in BALB/c mice transplanted with cells of the line CT26 exposed to high levels of glucose, to mimic a colorectal cancer. The sesquiterpene blocks ART1 effects, by inhibiting NF-κB. ART1 (arginine-specific mono-ADP-ribosyl transferase 1) is an enzyme, whose concentrations are higher in patients with type 2 diabetes, involved in the pathogenesis of colorectal cancer. The overexpression of ART1 probably increases glycolysis and energy metabolism, thus regulating the protein kinase B/mammalian target of rapamycin/c-Myc signaling pathway and the expression of glycolytic enzymes. This suggests that BCPO may be a potential treatment for this kind of carcinoma [51].

Further, BCPO is cytotoxic against various cell lines, including: HeLa (human cervical adenocarcinoma cells), HepG2 (human leukaemia cells), AGS (human lung cancer cells), SNU-1 and SNU-16 (human stomach cancer cells) and A-2780 (human ovarian cancer cells). It modulates many fundamental pathways in tumour pathogenesis, such as those involving MAPK, Phosphoinositide 3-kinases (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR)/S6 kinase 1 (S6K1) and Signal Transducer and Activator of Transcription (STAT3) [13].

BCPO, like BCP, shows synergy with antitumor drugs, like paclitaxel and doxorubicin. In particular, it is able to increase the concentration of the two drugs in many cancer cell lines, including CaCo-2.

Anyhow, between the two sesquiterpenes, the oxidised derivative has the greatest antitumor activity, variable according to cell types and dosages. In fact, due to the epoxide function, BCPO binds covalently to amino groups and thiol moieties of proteins and nitrogen bases, which constitute the nucleic acids. In any case, both the molecules achieve their antitumor action by causing apoptosis and blocking the cell cycle [13].

2.3. β-Caryophyllene and Inflammatory Diseases

Acute inflammation is a body defence mechanism against various factors, which may evolve in chronic inflammation, resulting in pathological disease [81]. After the acute reaction, monocytes remain in the inflammation site, where they secrete cytokines and chemokines and stimulate macrophages, amplifying the inflammatory response. As for neuroinflammation, the main phlogistic mediators are interleukins IL-1β, IL-6, and TNF-α, which increase NF-κB (nuclear factor kappa B) expression, prostaglandins and leukotrienes, synthesized by cyclooxygenases (COX) and 5-lipoxigenase (LOX) [82].

An in vitro study on macrophages of RAW267.4 [83] mice has revealed that BCP, administered in association to other two natural molecules, baicalin (**10**) and (+)-catechin (**11**) (Figure 4), at relatively low doses, suppresses the proliferation of these cells involved in inflammation [52].
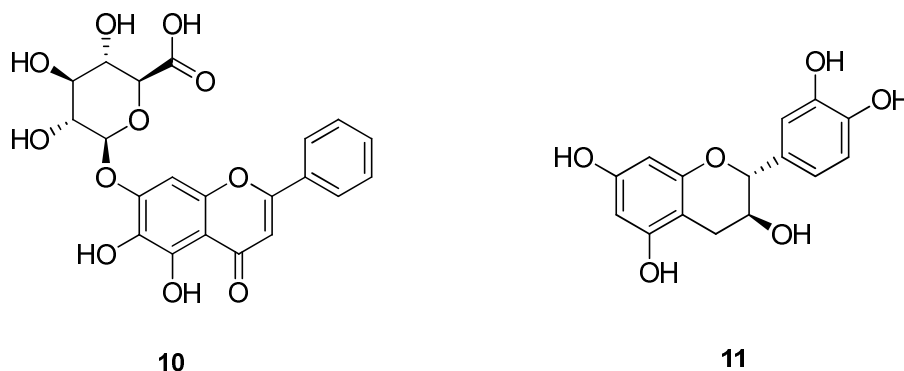

Figure 4. Structures of the baicalin (**10**) and (+)-catechin (**11**).

The three substances act synergically, since they separately do not exert any significant activity. Their effect is achieved through the cell cycle arrest phase G2/M and the modulation of various intracellular pathways, such as PI3K/Akt, extracellular signal-regulated kinases(ERK)/MAPK, and calcium homeostasis. In particular, the decrease of the expression of Akt, MAPK p38 and p44/42, and caspase-3 activation, an important step of apoptosis, have been observed. In addition to these effects, the expression of COX-1 and COX-2 diminishes, and so the activity of the protein p65 of NF-κB family does [52].

According to a recent study, BCP exerted powerful results against the negative effects of dyslipidemia and vascular inflammation in mice [53]. In fact, it has been revealed that the treatment with the sesquiterpene, at the dose of 30 mg/kg, prevents the increase of adiposity index, glycemia and insulinemia due to a high fat-diet. It also helps dyslipidemia and reduces all atherogenic risk indexes, even if it does not modify body weight. BCP reduces oxidative stress, by decreasing the concentration of NO and malondialdehyde (MDA), a by-product of lipid peroxidation, and by increasing the level of the endogenous antioxidant glutathione [84]. The phytocannabinoid is able to suppress mediators involved both in inflammation and atherosclerosis, such as TNF-α and NF-κB. In this way, it leads to the inhibition of VCAM1 [53], a vascular cell adhesion protein, which promotes the adhesion of white cells of the vascular endothelium and favours atherosclerosis, confirming what was reported in other in vitro researches [54].

BCP normalizes the ratio between endothelial (eNOS) and inducible (iNOS) nitric oxide synthase within the aorta. The latter is activated in the phlogistic process and in the atherosclerosis following the oxidative stress-induced NF-κB activation. Further, iNOS produces a high amount of nitric oxide, which interacts with ROS, generating peroxynitrites, which amplify the oxidative stress.

Furthermore, BCP attenuates the formation of foam cells and the deposition of collagen, which plays a crucial role in the formation and progression of vulnerable atherosclerotic plaques, and protects the integrity of elastic lamina.

All these effects are attributable to direct action of BCP, as agonist of cannabinoid receptors CB2, other than of PPAR-γ receptors (receptors activated by peroxisome proliferator-activated receptors), involved in the reduction of blood levels of total cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL), in the inhibition of vascular inflammation and synthesis of adhesion molecules and in the rebalancing of nitric oxide concentration and nitric oxide synthase isoforms. Moreover, it would seem that BCP is also an agonist of PPAR-α receptors, reduces fat mass and triglycerides and increases high density lipoprotein (HDL). This happens through the binding of BCP to cannabinoid receptors CB2-R, which activates PGC1-α (coactivator 1 of the peroxisome gamma proliferator) and allows the interaction among PPAR-γ and various transcriptional factors, such as PPAR-α, increasing the expression of enzymes with the function of oxidizing fat acids, above all in the liver.

The comparison with the thiazolidinedione pioglitazone (**12**) (Figure 5), a PPAR-γ agonist used for the treatment of type 2 diabetes and atherosclerosis, has shown that BCP is more effective than the drug for all the parameters, except for the glutathione levels. In addition, the sesquiterpene does not induce body weight gain, the main side effect of pioglitazone [53].
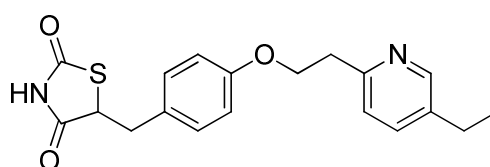
12

Figure 5. Structure of the pioglitazone.

BCP is beneficial in a model of bilateral carotid artery occlusion and reperfusion (BCCAO/R) in Wistar rats. This study has demonstrated that a single dose of BCP prevents plasmatic and

tissue modifications induced by carotid obstruction and reperfusion [55]. It increases tissue levels of endocannabinoids (anandamide, 2-arachidonoylglycerol, palmitoylethanolamide, oleylethanolamide) and cannabinoid receptors CB1 and CB2, reducing anandamide blood concentrations. Furthermore, it preserves the tissue levels of the essential fatty acid docosahexaenoic acid (DHA), increases PPAR-α expression, and decreases lipoperoxidation damage [55].

BCP effects have been studied in a rat model of rheumatoid arthritis, characterized by inflammatory response.

BCP and copaiba oil (the sesquiterpene accounts for about 37% of the oil) have the same ability to reduce paw edema, popliteal lymph nodes weight and myeloperoxidase plasmatic activity. BCP, unlike copaiba oil, decreases also the activity of hepatic myeloperoxidase and leukocytes, both the blood ones and those present in the joints. The anti-inflammatory activity is slightly greater in copaiba oil than in BCP, probably because of the presence of other molecules with synergist effect. Neither the oil nor the single molecule are able to modify secondary injuries and body weight in rats. Both are capable, at high doses, to:

- Reduce carboxylic groups of protein and ROS in the liver, normalizing the levels;
- Increase glutathione levels and reduce lipoperoxidation [56].

Copaiba oil, because of the presence of diterpenes, like kaurenoic acid and hardwickiic acid, is hepatotoxic, reducing the liver functionality due to hepatic cholestasis. In this context, the use of isolated BCP is preferable, since it is hepatoprotective [57].

BCP exerts in vitro [45,85,86] and in vivo antioxidant capacities.

As regards the mechanism, BCP, at a dose of 430 mg / kg, exhibits antioxidant activity and acts by exerting various functions: radical scavenging ability in particular with respect to hydroxyl radicals, lipid peroxides and superoxide anions; stimulation of the endogenous antioxidant system, highlighted by the increased content of glutathione in the liver, induced by Nrf. A decrease in inflammation, characterized by reduced activity of myeloperoxidase, is a marker of infiltration of polymorphonuclear cells, and diminished expression of COX-2 and cytokines, such as TNF-α, IL-1β, IL-6, and consequently, NF-κB.

Also, BCP effects on C57BL/6J mice have been investigated. The mice, fed with an essential amino acid-deficient diet, are a model of non-alcoholic steatohepatitis (NASH), which is a hepatic inflammatory pathology associated to metabolic syndrome predisposing cardio-vascular diseases. In the case of steatohepatitis, the organ undergoes histological changes caused by oxidative stress, inflammation and fibrosis. The treatment with BCP reduced inflammation and fibrosis. Moreover, a decrease in alanine-transaminase (ALT) and cytokine expression has been observed, suggesting that the liver has been less damaged. BCP exerts antioxidant effect, by increasing the levels of the enzymes SOD2 (superoxide dismutase 2) and GPx1 (glutathione peroxidase 1), both involved in free radical detoxification. In addition, the enzyme Nox2 (reduced form of nicotinamide adenine dinucleotide phosphate (NADP) oxidase 2), TGF-β, and collagen, all elements which contribute to hepatic fibrosis, have been inhibited by the sesquiterpene [58].

2.4. β-Caryophyllene and Micro-Organisms

BCP has antimicrobial activity both against Gram-positive bacteria, such as *Staphilococcus aureus* and Gram-negative bacteria, including *Escherichia coli* [59]. It is more effective against Gram-positive and the activity of *Artemisia fed dei* extracts, containing BCP, against bacteria responsible for tooth decay and periodontitis has been reported [60]. The antimicrobial activity of the essential oil and some of its compounds was tested against 15 different genera of oral bacteria, including *Streptococcus mutans*, which produces a biofilm, constituted by a variety of extracellular polymeric substances (EPS), which protect micro-organisms of oral cavity. The antibacterial activities of the essential oil and of BCP was determined based on the minimum inhibitory concentrations (MICs) which were determined as the lowest concentration of the test samples that resulted in a complete inhibition of visible growth in

the broth. BCP kills *Streptococcus mutans* with a minimum inhibiting concentration (MIC) of 0.32% by penetrating the biofilm, which constitutes a protective barrier against external substances, acting as an ion exchange resin. *Streptococcus mutans* uptakes sucrose, which is necessary for glucan synthesis, through the enzymes GtfB, GtfC and GtfD. The first two are required for the synthesis of insoluble glucans, which facilitate bacterial aggregation. GtfD, on the other hand, promotes the synthesis of water-soluble glucans, which are necessary for biofilm development. The mechanism of action of BCP is to reduce the expression of Gtf genes, thus preventing biofilm synthesis.

2.5. β-Caryophyllene and Osteoporosis

BCP exerts effects on various tissues, like bone tissue. In fact, it stimulates the differentiation of multipotent stem cells (MSC) of bone marrow (which can differentiate in many cell types, including adipocytes, osteoblasts, myoblasts, chondrocytes, etc.) in osteoblasts, limiting the formation of adipose cells. In order to modulate this process, regulated by various pathways, BCP activates PPAR-γ. It suppresses osteoclastogenesis, which is the synthesis of osteoclasts, cells derived from haematopoietic progenitors which promote bone resorption. This mechanism, TNF-α-mediated, activates the transcription factor NF-κB in prosteoclasts and it is inhibited in vitro by BCP (in cell cultures of murine bone marrow) in the differentiation stage in prosteoclasts.

Therefore, BCP enhances osteoblast mineralization, favouring osteoblastogenesis and suppressing osteoclastogenesis and adipogenesis, which is an interesting property for the potential application in the treatment of osteoporosis, in particular the one associated to obesity and diabetes [61].

2.6. β-Caryophyllene Toxicity

BCP is classified according to OECD (Organization for Economic Co-operation and Development) guideline 423 as a category five substance (toxic at doses greater than 2000 mg/kg) [65].

Studies about acute toxicity have been carried out. The oral administration of 2000 mg/kg of the molecule in female Swiss mice induces no toxic effects [10], whereas in rats an LD_{50} greater than 5000 mg/kg has been calculated [62].

Subchronic toxicity (90 days) has been assessed in studies in Wistar rats, which do not show toxicity at the dose of 700 mg/kg/die [62], and in female Swiss mice, which are not altered in terms of locomotion and muscle tone with both single and repeated doses [10].

In rodents, overall absence of neurotoxicity at the dosages used to study pharmacological effects (20 to 100 mg/kg) has been found [10,41]. Moreover, in various studies, no damage to the gastric mucosa has been observed, nor changes in other internal organs (brain, heart, liver, lungs, spleen, kidneys) or in haematological parameters have been reported both in female Swiss mice and in Wistar rats [10,62]. Moreover, an Ames test has shown no mutagenicity [63].

Body weight has resulted decreased by 5% in female Swiss mice, even if this variation is not significant [10]. Nevertheless, even though this molecule is safe, at least in animal studies, greater consideration is needed of the possible interactions of the sesquiterpene with drugs and other molecules contained in the food. In fact, a research conducted on subcellular fractions of hepatic tissue of rats and humans, demonstrates a significant inhibition by BCP and its derivatives BCPO and α-humulene against enzymes involved in metabolism and xenobiotic detoxification. Xenobiotics undergo metabolic biotransformations, followed by degradation and subsequent elimination. It is evident that the inhibitory effects exerted by BCP on drug metabolizing enzymes considerably increases the levels of drugs in the organism, with prolonged duration of action and increased toxicity. This makes absolutely necessary a variation of the posology of drugs taken at the same time of enzymatic inhibitors. The extent of the inhibition of cytochrome P450 isoform CYP1A2 (Cytochrome P450 Family 1 Subfamily A Member 2) is greater in rat microsomes than in human ones and, above all, BCPO is able to competitively inhibit the enzyme in rats and humans through a non-competitive mechanism. However, BCP and α-humulene result less powerful than BCPO [64].

Furthermore, they have a fair capability to inhibit CYP3A4, involved in the metabolism of many xenobiotics, such as ciclosporin, paclitaxel, ketoconazole, verapamil, nifedipine, omeprazole, statins, various sexual hormones and drugs. The inhibition causes the increase of medicine levels, which could determine severe side effects. Finally, the three terpenes exert a weak inhibition against the isoforms CYP2A6 (Cytochrome P450 Family 2 Subfamily A Member 6), CYP2B6 (Cytochrome P450 Family 2 Subfamily B Member 6), CYP2C9 (Cytochrome P450 Family 2 Subfamily C Member 9), CYP2C19 (Cytochrome P450 Family 2 Subfamily C Member 19), CYP2D6 (Cytochrome P450 Family 2 Subfamily D Member 6), CYP2E1 (Cytochrome P450 Family 2 Subfamily E Member 1) [64].

3. Conclusions

BCP is a plant compound which has been demonstrated to possess a great potential application for various pathological conditions, due, above all, to the selectivity towards CB2 receptors, which, in addition to making this sesquiterpene devoid of psychogenic effects typical of cannabinoids, determines its main biological effects. In fact, BCP contrast in the animals the inflammatory process, typical of various degenerative diseases, which involve central nervous system (Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, etc.), steatohepatitis, osteoporosis, but also cancer and *Streptococcus mutans* infections.

However, even if the studies on the molecule are very promising, these are only preclinical (in vitro or in vivo in animal models) and further insights and clinical trials are required for a future human application.

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