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IDENTIFICATION AND STRUCTURAL ELUCIDATION OF 3-BROMO-10-CAMPHOR SULPHONIC ACID IN *CUCUMIS TRIGONUS* ROXB

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ABSTRACT

The fruit extract of *Cucumis trigonus* Roxb of family Curcurbitaceae was subjected to isolation and identification of chemical constituents. The extract was purified and isolated by column chromatography and thin layer chromatography (TLC). The isolated compound was then subjected to Infra red spectroscopy (IR) for identification of functional groups, ^{1}H NMR and ^{13}C NMR for identification of protons and carbon atoms. LCMS and elemental analysis were done to identify the molecular weight and elemental composition of the isolated compound. From the spectra obtained from IR, ^{1}H NMR and ^{13}C NMR, LCMS and elemental analysis, the isolated compound was found to be 3-Bromo -10- camphor sulphonic acid ($C_{10}H_{17}BrO_5S$) with a molecular weight of 329.

KEY WORDS

Cucumis trigonus, Thin layer chromatography, Infra red spectroscopy, 3-Bromo-10-camphor sulphonic acid

INTRODUCTION

Plants are utilized as therapeutic agents since time immemorial in both organized and unorganized form. The healing properties of many herbal medicines have been recognized in many ancient cultures[1]. Plants provide a variety of resources that contribute to the fundamental needs of food, clothing and shelter. Among plants of economic importance, medicinal and aromatic plants have played a vital role in alleviating human sufferings [2].

The recent resurgence of plant remedies results from several factors like effectiveness of plant medicines, lesser side effects and even supplementation compared to modern medicines [3]. In the present scenario, the need for basic scientific investigations on medicinal plants used in the indigenous systems becomes imminent. This is evident by the increase in number of reports by various investigators supporting the claims of medicinal plants and a dramatic increase in the share of plant products in pharmaceutical market [4].

Plants have the ability to synthesize secondary metabolites to defend them against their predators. Some of these compounds turn out to have beneficial effects towards human diseases[5]. Secondary metabolites are highly varied in structure, many are phenolic aromatic substances or oxygen substituted derivatives[6]. Many herbs and spices used by humans yield useful medicinal compounds [7].

Numerous molecules have come out of Ayurvedic experiential base. Examples include rauwolfia alkaloids for hypertension, holarrhena alkaloids in amoebiasis, guggulsterons as hypolipidemic agents, mucuna pruriens for Parkinson's disease, piperidines as bioavailability enhancers, baccosides in mental retention, picrosides in hepatic protection, phyllanthins as antivirals, curcumine in inflammation, many other steroidal lactones and glycosides as immune-modulators. A whole range of difficult to treat diseases such as cancers, cardiovascular diseases, diabetes, rheumatism and AIDS require new effective drugs. Most developing countries have relied and will continue to rely on traditional natural medicines due to the high cost of modern allopathic medicines[8].

The plants used in the Indian system of medicine are of interest to find new leads for treating different diseases. Approaches like high-throughput screening, phytochemical profiling, quality controls and standardization of raw materials and finished products, clinical trials, herbal therapeutics, pharmacokinetics and herbal pharmacovigilance will not only help to prove the rationale of using these systems but also to get maximum benefits of the natural resources [9].

Cucumis trigonus Roxburghii of family Curcurbitaceae is a perennial scabrid monoecious tendrillar herb with slender angled stem, leaves deep palmately five lobed, hispid on the nerves beneath and rounded at the apex. Male flowers are small and are found in clusters whereas female flowers are solitary. Fruits are ellipsoid or sub-global, yellow or yellow with green stripes and seeds are white and ellipsoid [10]. The plant is distributed throughout India and found in areas of Ceylon, Afghanistan, Persia and Northern Australia[11]. Roots, fruits and seeds are extensively used medicinal parts of the plant. Roots are purgative and liver tonic. Fruits are used for stomachic, ascites, anemia and constipation and acts as a diuretic. Seeds have

unsaturated lipids as major constituents and acts as a coolant and astringent[12].

The present study deals with the extraction and characterization of ethanolic fruit extract of *Cucumis trigonus*. The characterization of the extract includes the isolation and purification using the column and thin layer chromatography. The isolated compounds from TLC were subjected to various instrumental analysis like IR, ^{1}H NMR and ^{13}C NMR were done to identify the presence of functional groups, protons and carbon atoms respectively. LCMS and elemental analysis were done to identify the molecular weight and the elemental composition of the isolated compound.

MATERIALS AND METHODS

Extraction350g of the dried fruits of *Cucumis trigonus* was soaked in 1L ethanol and allowed to stand for 24 hours filtered the solvent and extraction was repeated thrice by adding 1L of ethanol each time. Then all the three extracts were collected and concentrated using rotary vacuum to get 20g of crude extract.

Isolation, purification and identification

i) Column chromatography

20g of the crude extract was redissolved in minimum quantity of ethanol. To this 60g of silica gel (60:120 Mesh) was added and allowed to dry to get free flow of admixture. This admixture was packed for column chromatography and column was eluted using increasing polarity of solvent as mentioned in table 1.

Table1
Column chromatographic solvent systems for the separation of active constituents

S.No	% of solvents	Fraction number	Vol. of solvent
1	100%Hexane	1-6	300
2	5%E.Ac/hexane	7-16	500
3	10%E.Ac/hexane	17-24	400
4	15%E.Ac/hexane	25-34	500
5	20%E.Ac/hexane	35-44	500
6	25%E.Ac/hexane	45-50	300
7	30%E.Ac/hexane	51-58	400
8	35%E.Ac/hexane	59-64	300
9	40%E.Ac/hexane	65-70	300

E.Ac= Ethyl acetate

Fraction 55th- 68th were combined and further treated with charcoal to get the pure compound.

The compounds obtained from column chromatography were separated and concentrated. The compounds were then tested using TLC for identification.

ii) Thin layer chromatography (TLC)

In this process, we coated the slurry (1:2) over the glass plates at a thickness of 0.25mm and allowed to dry at room temperature for 15-30 min. The plates were heated in an oven at 100-120°C for 1-2 hr. to remove the moisture and to activate the adsorbent on the plate. The column eluted was applied at 2.5cm from one end of the glass plate and allowed the samples to dry so that spotting can be done repeatedly for a more concentrated samples spot. The developing solvent was poured into a tank till a depth of 1.5cm. After equilibration, the cover plate was removed and the thin layer plate (sample applied) was placed vertically in the tank so that it stands in the solvent with the spotted end dipping in the solvent. The separation of the compounds occurs as the solvent moves upward. Once the solvent reaches the top of the plate, it was removed from the tank, dried and sprayed with the spraying reagent for the identification of the separated compounds. The samples produce colour with the spraying agent.

iii) Infra red (IR) spectral studies

The infrared spectrum originates from the vibration motion of the molecule. The vibration frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is also carried out by using Fourier transform (FT) technique.

The interference pattern obtained from a two beam interferometer as the path difference between the two beams is altered, when Fourier transformed, gives rise to the spectrum. The transformation of the interferogram into spectrum is carried out mathematically with a dedicated on-line computer.

The Shimadzu Spectrum1 FT-IR instrument consists of globar and mercury vapour lamp as sources, an interferometer chamber comprising of KBr and mylar beam splitters followed by a sample chamber and detector. Entire region of 450-4000 cm^{-1} is covered by this instrument. The spectrometer works under purged conditions. Solid samples are dispersed in KBr or polyethylene pellets depending on the region of interest. This instrument has a typical resolution of 1.0 cm^{-1}. Signal averaging, signal enhancement, base line correction and other spectral manipulations are possible.

iv) ^{1}H NMR and ^{13}C NMR spectral studies

FT-NMR spectroscopy is used to determine the molecular structure based on the chemical environment of the magnetic nuclei like ^{1}H, ^{13}C, ^{31}P, etc., even at low concentrations. This is one of the most powerful nondestructive techniques in elucidating the molecular structure of biological and chemical compounds.

In FT NMR spectroscopy, a strong RF pulse excites the entire range of processional frequencies of a given nuclear species whose time response is known as free induction decay (FID) containing all the information. A Fourier transform of FID gives the NMR spectrum. This is a much faster technique compared to continuous wave and hence it is possible to detect weak lines by signal averaging methods. This technique is used in JEOL, GSX 400 NB FT-NMR spectrometer. The spectra of samples containing low abundant nuclei like ^{13}C, ^{31}P, etc., are thus easily obtained. Also dynamic studies are possible by relaxation measurements. Homo and hetero ^{1}H decoupling are also possible.

JEOL GSX 400 NMR operates at 400 MHz (for proton) with a magnetic field of 9.3

Tesla. Hence a supercon magnet is used. A PDP-11/73 computer is an integral part of the instrument for the purpose of Fourier transformation and spectral manipulation. The spectrum is plotted on a HP plotter and data can be obtained on a printer. The probes available are $^{1}H/^{13}C$ combined and multinuclei probe to study the nuclei like ^{23}Na, ^{27}Al, ^{43}Ca, ^{37}Cl, ^{79}Br, etc., except ^{19}F, ^{3}H and T_1. Among other things, many types of 2D spectral measurements are possible. It is possible to obtain high resolution NMR spectra of many compounds using the solids accessory employing MAS technique which otherwise are insoluble in usual solvents. Though the resolution is poor, this is also useful for polymers, etc.

Sample required are 5 mg for ^{1}H and 15mg for ^{13}C and other nuclei. Solubility is 10mg / ml for ^{1}H and 50 mg / ml for ^{13}C and others. Solvent must be specified and solubility must be checked before injecting the samples in order to save the expensive deuterated solvents. Sample must be free of paramagnetic impurities. Solvents available: $CDCl_3$, D_2O, C_6H_6, CD_3COCD_3 and $DMSOd_6$.

v) Determination of molecular weight of the compound by liquid chromatography mass spectroscopy (LC MS)

Mass spectrometry has become a vital tool in the hands of organic chemists and biochemists because of its potential to supply definitive, qualitative and quantitative information on molecules based on their structural compositions.

The mass spectrometer consists of an ion source, an analyzer and a detector maintained at a vacuum of 10^{-8} torr. The vaporized molecules are first bombarded by a stream of high energy electrons converting some of the molecules into molecular ions and fragment ions. The ions are accelerated and separated according to their mass to charge ratios in the magnetic field (analyzer). These are then velocity focused in an electric field. The ions are detected in terms of their mass to charge ratios by the detector namely a secondary electron multiplier. The output is amplified and fed to the recorder for processing. The mass spectrum, a graph of intensity of the ions detected vs. m/z value is presented on the screen and printed. An IBM compatible PC is used to control the Mass spectrometer and also to acquire process and print out the spectral data.

The 410 Shimadzu Binary LC 500 Mass spectrometer with data system is a high resolution, double focusing instrument with reverse Nier-Johnson geometry. The maximum resolution is 48000 at 10% valley in low resolution mode. Maximum calibrated mass is 2000 Daltons.

Sample required is about 1 mg which can be in the solid or liquid state. Sample should be pure and free from solvents and metal ions.

vi) Identification of elements by elemental analysis

The carbon, hydrogen, nitrogen and oxygen contents of the compounds were analyzed by Erlinmeier flask method.

vii) Structural elucidation

The spectra of IR, ^{1}H NMR and ^{13}C NMR, elemental analysis and LC MS were used for structural elucidation of the isolated compounds.

RESULTS AND DISCUSSION

Compound code: BRK-4
Colour of the compound: White
Melting point: 117-121°C

1. Identification of functional group by IR spectral studies

The IR spectrum of the isolated compound BRK-4 showed peaks at 3554.93 cm^{-1} and 3448.84 cm^{-1}, which indicates the presence of H_2O and OH group. Another peak at 1622.19 cm^{-1} indicates the presence of carbonyl (C=O) group and the peak at 1099.46 cm^{-1} is

registered for the presence of S=O group. The spectrum is shown at figure 1.

2. Structural elucidation of active constituent by ^{1}H NMR and ^{13}C NMR spectral studies

^{1}H NMR spectrum of the isolated compound BRK-4 shows two multiplets in the region δ 0.9 - 1.00 for C_5 and C_6 protons and δ 1.31 – 1.50 for C_3 and C_4 protons. The spectrum also registered three singlets at δ 1.30, δ 3.20 and δ 3.60 for methyl, methylene and OH groups respectively.

^{13}C NMR showed the peak at δ 164.5 to represent the carbonyl (C=O) group at C_2. Peaks representing at δ 22.6 and δ 27.6 are for methyl carbons. The other peaks shown at δ 47.9, δ 64.5, δ 60.2, δ 54.6, δ 36.2, δ 39.2 and δ 60.9 represent C_1 to C_7. The methylene group (CH_2) at C_{10} is represented by the peak at δ 39.2. The ^{1}H NMR and ^{13}C NMR spectra are shown in the figures 2 and 3.

3. Identification of active constituent by mass spectrum

The mass spectrum showed ion peak m /z at 329 and mass fragmentation ion peaks at 217, 280 and 290. The mass spectrum is shown in figure 4.

4. Identification of active constituent by elemental analysis

The elemental analysis of the isolated compound BRK-4 is given as a graphical representation in the figure 5. The elemental analysis showed the presence of 36.48% carbon, 5.2% hydrogen, 9.72% sulphur, 24.27% bromine and 24.3% oxygen.

From the mass spectrum and the elemental analysis of the isolated compound, the molecular weight was confirmed as 329 and molecular formula was $C_{10}H_{17}BrO_5S$ and the structure elucidated is given in figure 6.

All our spectral data coincide with the spectral data of 3-bromo -10- camphor sulphonic acid, which confirms that the isolated compound is 3-bromo -10- camphor sulphonic acid

Figure .1
IR Spectra of the isolated compound

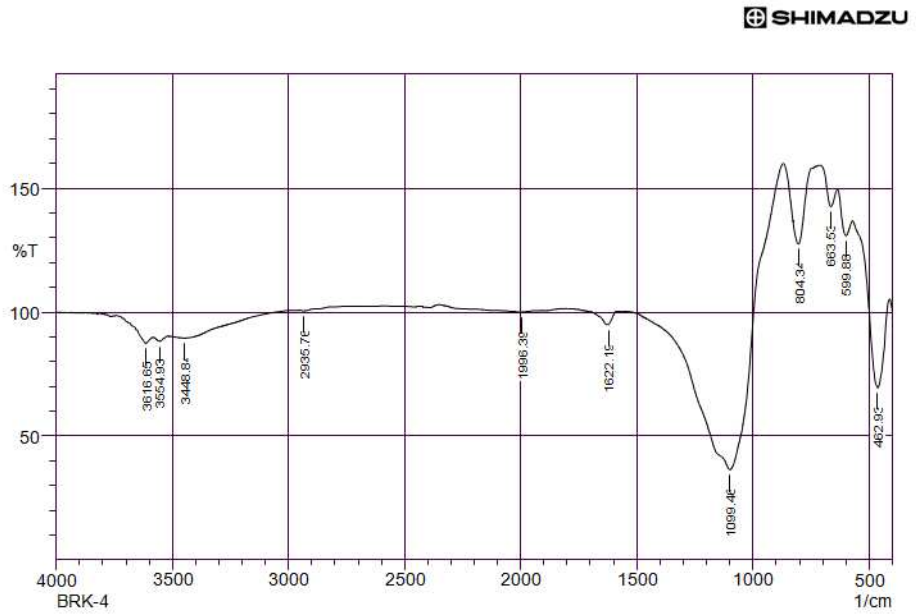


Figure .2
. ^{1}H NMR spectra of the isolated compound 2

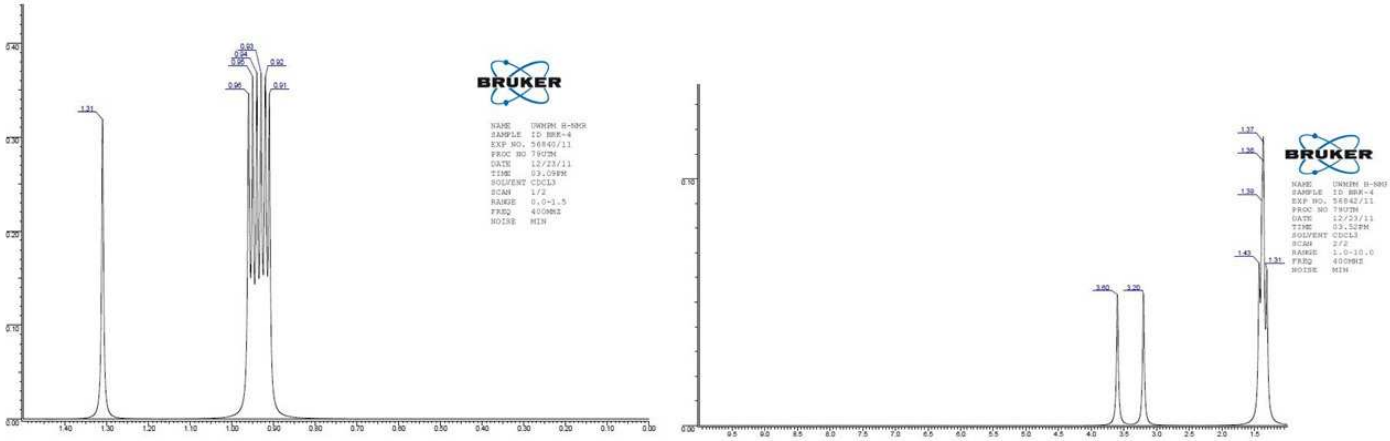


Figure .3.
^{13}C NMR Spectrum of the isolated compound 2

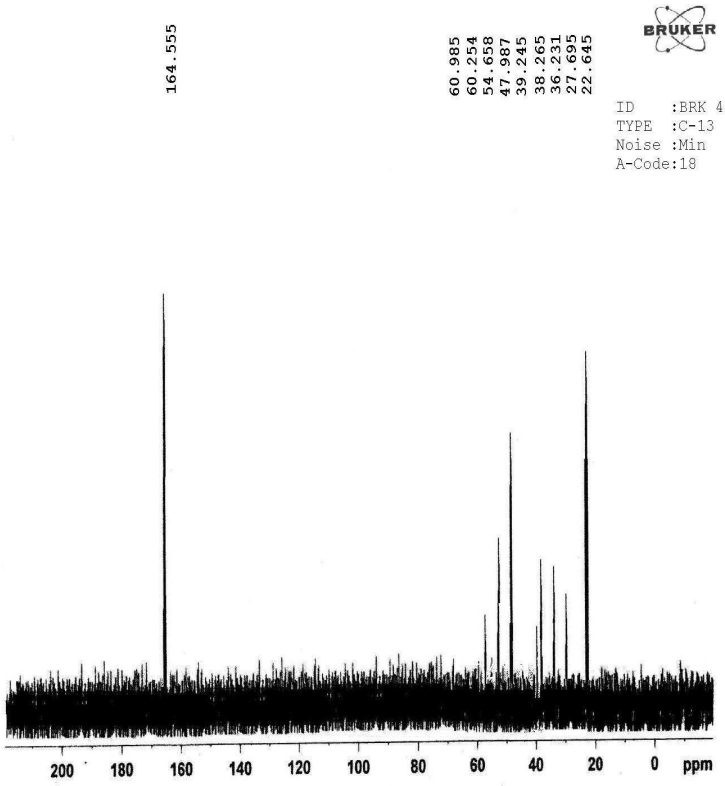
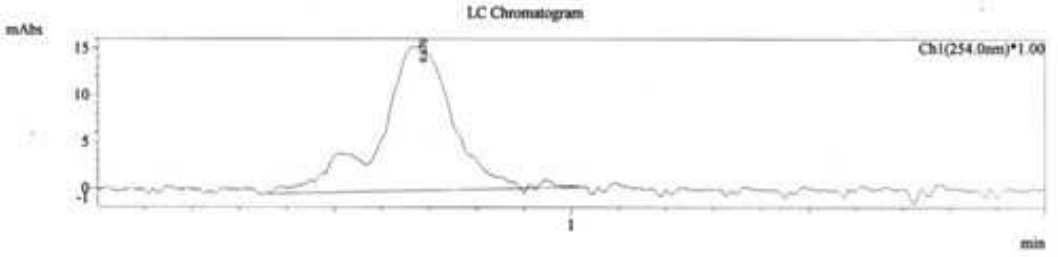


Figure .4.
Mass spectrum of the isolated compound 2

LCMS-2010A DATA REPORT
SHIMADZU

User : Admin
Sample : BRK4
Inj. Volume : 5.000
Data Name : C:\LCMSsolution\User\Data\BRK4-APCI-NEG1.qld
Method Name : C:\LCMSsolution\User\Method\esi.qlm



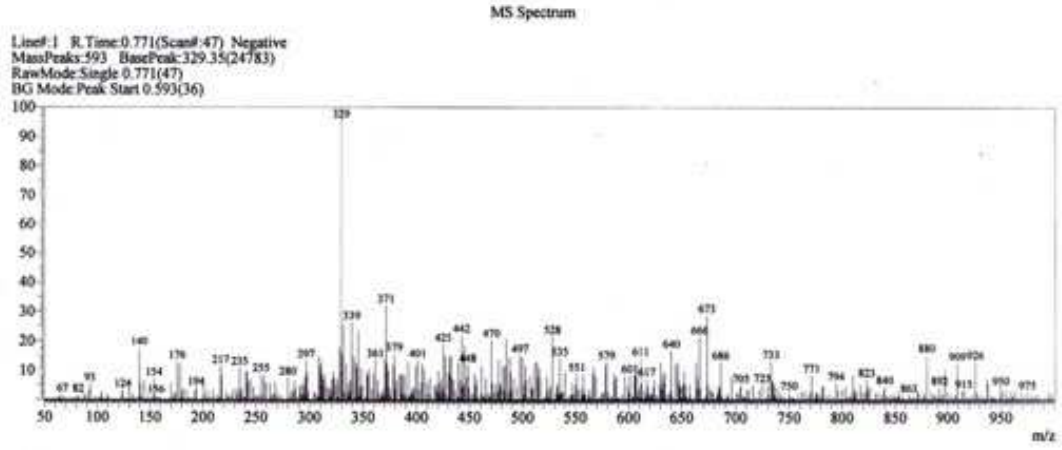
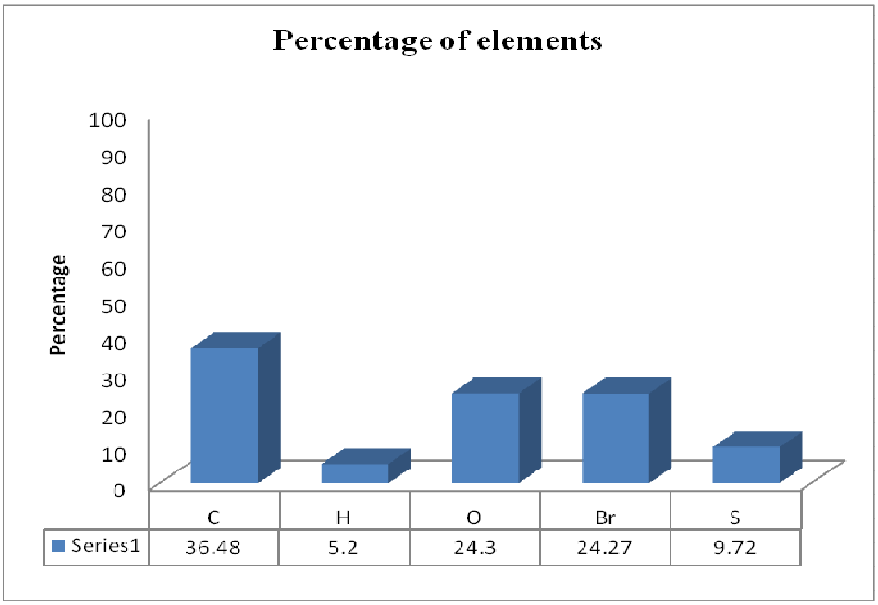


Figure .5.
Elemental analysis of the isolated compound 2



Molecular weight: 329
Molecular formula: $C_{10}H_{17}BrO_5S$

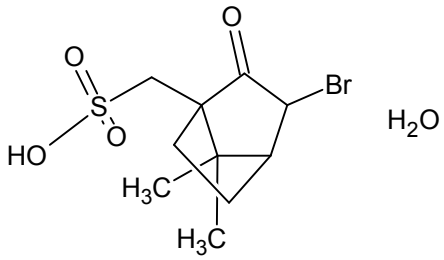


Figure .6.
Structure of the isolated compound 2

Compound Name: 3-Bromo-10-camphorsulfonic acid ;(3-bromo-7, 7-dimethyl-2-oxobicyclo [2.2.1] hept-1-yl) methanesulfonic acid.

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