as the chromatographic solvent. Then 200 g. of the chloroform extract of *A. oregona* was adsorbed onto 500 g. of alumina III, and the dried pulverized mixture was placed on top of the column. The column was eluted first with hexane and then with solvents of increasing polarity. Each fraction was examined by TLC, silica gel G, using chloroform–benzene–ethyl acetate (4:8:1) as the solvent system and ceric sulfate as the developing reagent. The fractions showing the same materials on TLC were combined into six groups.

Isolation of Lupeol—Fraction C yielded a large precipitate of crystals. After repeated crystallizations from chloroform–methanol, 14 g. of a colorless crystalline compound was obtained. Its melting point was 210–212°, $[\alpha]_D$ +25°, compared with the values for lupeol of 215° and $[\alpha]_D$ +27° (10).

Preparation of Lupeol Acetate—Lupeol (0.138 g.) was dissolved in 2.0 ml. of pyridine, and 2.0 ml. of acetic anhydride was added to the mixture. The mixture was left standing overnight at room temperature. It was then poured over crushed ice. The precipitated compound was collected and washed thoroughly with water until free of acetic acid and pyridine. After drying under vacuum, the compound was recrystallized twice from chloroform–methanol. Solid needles of crystals, m.p. 211–212° and $[\alpha]_D$ +33°, were obtained. The reported values for lupeol acetate were m.p. 218° and $[\alpha]_D$ +47° (10).

Preparation of Lupeol Benzoate—A mixture of isolated lupeol (200 mg.), pyridine (2.0 ml.), and benzoyl chloride (2.0 ml.) was refluxed for 1 hr. It was then cooled and poured over crushed ice. The mixture was refrigerated for 1 day. The precipitated compound was filtered off and then redissolved in ether. The ethereal solution was successively washed three times each with 5% sodium bicarbonate, 5% hydrochloric acid, and water. After drying the washed ethereal layer over anhydrous sodium sulfate, it was filtered and the ether was allowed to evaporate at room temperature. The residue, upon repeated crystallization from chloroform–ethanol, gave long platelike crystals, m.p. 258–260°, $[\alpha]_D$ +56° [lit. (10) m.p. 273–274°, $[\alpha]_D$ +61°]. The IR spectrum of this compound (KBr pellet) and that of an authentic sample of lupeol benzoate were superimposable.

Isolation of Betulin—Fraction F yielded a large quantity of a crystalline compound. This material was purified by recrystallization from chloroform and then from ethanol. Solid, thin, rodlike crystals, m.p. and mixed m.p. 253–254°, $[\alpha]_D$ + 18° [lit. (11) m.p. 251–252°, $[\alpha]_D$ +20°] were obtained. The IR spectrum of the isolated betulin (KBr pellet) and that of an authentic sample of betulin were in complete agreement.

Preparation of Betulin Diacetate—Betulin (0.3 g.), pyridine (0.3 ml.), and acetic anhydride (3.0 ml.) were refluxed for 1 hr. After cooling the reaction mixture, a precipitate was obtained. The material, after repeated crystallization from ethanol, gave bladelike crystals, m.p. 222–225°, $[\alpha]_D$ +24° [lit. (10) m.p. 223–224°, $[\alpha]_D$ +22°]. An authentic sample of this compound gave an identical IR spectrum (KBr pellets).

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▲ To whom inquiries should be directed.

Antibacterial Activity of Certain 3-Substituted Benzothiazoline-2-thiones

RAJENDRA S. VARMA, SYED A. IMAM*, and W. LEWIS NOBLES▲

Abstract □ Preliminary antibacterial screening results for 18 compounds are provided. Sixteen compounds exhibited some degree of activity.

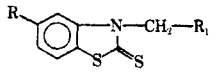
Keyphrases □ Benzothiazoline-2-thiones, 3-substituted—18 compounds screened for antibacterial activity against four organisms □ Antibacterial activity—18 3-substituted benzothiazoline-2-thiones screened against four organisms □ 3-Aminomethylbenzothiazoline-2-thiones, substituted—18 compounds screened for antibacterial activity against four organisms

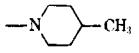
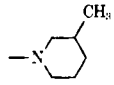
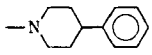
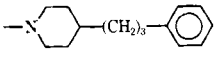
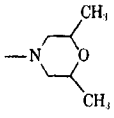
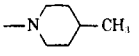
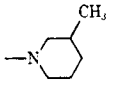
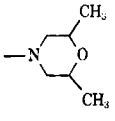
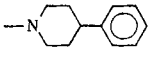
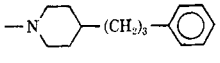
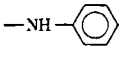
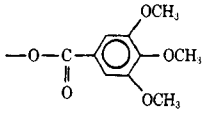
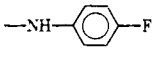
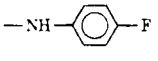
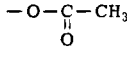
Antibacterial (1–4), antispasmodic (5), and antitubercular (6, 7) properties have been demonstrated by benzothiazoline-2-thione and some of its derivatives. Many of these compounds were effective against *Candida albicans* and bacterial strains resistant to penicillin (4). These observations led us to synthesize a series of 3-substituted benzothiazoline-2-thiones (I). The synthesis of I was reported elsewhere (8). The present article describes the evaluation of compounds of type I against four organisms by the agar diffusion technique (9).

EXPERIMENTAL

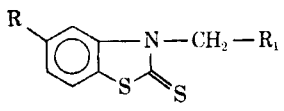
The agar medium was inoculated with 1 ml. of 24-hr.-old culture of the test organism. Filter paper disks (5-mm. diameter) saturated with the solution of the test compound (20 mg./ml. in ethanol) were

Table I—Antibacterial Activity of 3-Substituted Benzothiazoline-2-thiones



Compound	R	R_1	Microbial Spectrum[a] *Escherichia coli*	*Salmonella typhosa*	*Aerobacter aerogenes*	*Staphylococcus aureus*
1	H		−	−	+	−
2	H		++	++	+	+
3	H		−	—[b]	−	−
4	H		+	+	+++	−
5	H		+	++	+	−
6	Cl		+	−	—[b]	+++
7	Cl		+++	−	+	+++
8	Cl		++	+	+	+++
9	Cl		−	+	−	+
10	Cl		+	++	++	++
11	Cl		++	+	−	+++
12	Cl		+	−	+	−
13	H		−	−	−	−
14	Cl		+	−	−	−
15	H		−	−	−	+
16	Cl		−	−	−	+
17	H	−OH	++	++	+	+
18	Cl	−OH	+	+	+	+++

[a] Cultures maintained at Central Drug Research Institute, Lucknow, India, were used. Zone size: 6–10 mm. (+), 11–15 mm. (++), and greater than 15 mm. (+++). [b] No study was made in this case.



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placed on the agar after drying up the ethanol. After an incubation period of 36 hr., the zones of inhibition around the disks were measured. The organisms included in this study were: *Escherichia coli*, *Salmonella typhosa*, *Aerobacter aerogenes*, and *Staphylococcus aureus*.

DISCUSSION

Eight compounds, obtained by varying the substituents at the 3- and 5-positions of benzothiazoline-2-thione, were evaluated for their inhibitory effects on four organisms. The results of this study are recorded in Table I. The substituents at position 3 were: 3-methylpiperidinomethyl, 4-methylpiperidinomethyl, 4-phenylpiperidinomethyl, 4-phenylpropylpiperidinomethyl, 2,6-dimethylmorpholinomethyl, hexamethyleneiminomethyl, anilinomethyl, 4-fluoroanilinomethyl, acetoxymethyl, 3,4,5-trimethoxybenzoxymethyl, and hydroxymethyl groups. Position 5 was occupied by a chlorine atom in certain compounds, and others were without any substituents. Compounds 3 and 13 were devoid of any inhibition. Activity was considerably reduced when the heterocyclicamino function was replaced by the arylamino, acetoxy, or 3,4,5-trimethoxybenzoxy groups. Five compounds (2, 8, 10, 17, and 18) were active against all four organisms.

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* Present address: Division of Microbiology, Central Drug Research Institute, Lucknow, India.

▲ To whom inquiries should be directed. Present address: Office of the President, Mississippi College, Clinton, MS 39058

Viscosity Change in Salicylic Acid–Cetrimide System by Surfactants

LUCY S. C. WAN

Abstract □ The series of cationic and nonionic surfactants studied were found to decrease the viscosity of the salicylic acid–cetrimide system. This viscosity decrease varied with the surfactant concentration and with the volume of additive solution. With cationic surfactants, the fall in viscosity could be attributed to a dilution of the system, with the same type of surfactant resulting in a decrease of saturation; with nonionic surfactants, the fall in viscosity could be attributed to a penetration of the additive into the mesh structure of the macromolecules, bringing about a separation of the macromolecules from each other.

Keyphrases □ Viscosity—effect of surfactants on salicylic acid–cetrimide system □ Surfactants, cationic and nonionic—effect on viscosity of salicylic acid–cetrimide system □ Salicylic acid cetrimide system—effect of surfactants on viscosity □ Cetrimide–salicylic acid system—effect of surfactants on viscosity

As stated in an earlier paper (1), the anionic surfactants studied caused an increase in viscosity followed by a decrease in similar salicylic acid–cetrimide systems. It was considered logical as a follow-up to investigate the other two common types of surfactants as additives to these systems. Hence, some cationic and nonionic surfactants were selected on the basis that they could be used in pharmaceutical practice. Studies of this nature have not apparently been undertaken, although some related work has been carried out on the interaction of pharmaceutical compounds such as starch, amylopectin, and chondroitin with quaternary ammonium compounds (2–4).

EXPERIMENTAL

Materials—The cationic surfactants used were dodecyltrimethylammonium bromide[1], alkyl aryl trimethylammonium chloride[1], and cetrimide BP[2]; the nonionic surfactants were polysorbate 20[3], polysorbate 40[3], polysorbate 60[3], polysorbate 80[3], polysorbate 85[3], and polyoxyethylene ether of cetyl alcohol[4]. The recrystallized salicylic acid, m.p. 158–159°, was the same as that stated in a previous paper (5).

Measurement of Viscosity at 25°—A system containing 1.4% salicylic acid and 5% cetrimide in water was prepared, and its viscosity in the presence of an added solution of surfactants was

[1] Marketed as Morphan D and Gloquat C, respectively, by Glovers Chemicals Ltd., Leeds 12, England. The active ingredient content of Gloquat C is 50% w/w.

[2] Glovers Chemicals Ltd., Leeds 12, England.

[3] Marketed as Tween 20, 40, 60, 80, and 85, respectively, by Honeywill-Atlas Ltd., London, England.

[4] Marketed as Texofor A24 by Glovers Chemicals Ltd., Leeds 12, England.