Table 3 *Minimum* MSE's *and optimum weights of* $\bar{y}_s$

Sampling Schemes	Minimum MSE's	Optimum weights
1.	$M_0(\bar{y}_s) = \bar{Y}_0^2(\delta - \delta_0)/\delta_0$	$W_{10} = R_1\delta_1/\delta_0, W_{20} = R_2\delta_2/\delta_0$
2.	$M_0^*(\bar{y}_s) = \bar{Y}_0^2(\delta' - \delta_0)/\delta_0$	$W_{10} = R_1\delta_1 f/\delta_0, W_{20} = R_2 f\delta_2/\delta_0$
3.	$M_0^{**}(\bar{y}_s) = \bar{Y}_0^2(\delta'' - \delta_0')/\delta_0'$	$W_{10} = R_1 f\delta_1'/\delta_0'$, $W_{20} = R_2 f\delta_2'/\delta_0'$,

Table 4 *Difference of minimum* MSE's *of* $\bar{y}_d$ *and* $\bar{y}_s$

Sampling Schemes	Difference of Minimum MSE's
1.	$M_0(\bar{y}_s) - M_0(\bar{y}_d) = \bar{Y}_0^2(\delta - \delta_0)^2/(\delta\,\delta_0) > 0$
2.	$M_0^*(\bar{y}_s) - M_0^*(\bar{y}_d) = \bar{Y}_0^2(\delta' - \delta_0)^2/(\delta'\,\delta_0) > 0$
3.	$M_0^{**}(\bar{y}_s) - M_0^{**}(\bar{y}_d) = \bar{Y}_0^2(\delta'' - \delta_0')^2/(\delta''\,\delta_0') > 0$

Remarks

1. Following the procedure opted by Singh[4] one can easily obtain the conditions under which one sampling scheme provides better estimators than another one.
2. The results given for two auxiliary characters may also be generalized to p (> 2) auxiliary characters.

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SPASMOLYTIC AND ANTIOXYTOCIC COUMARINS FROM *HERACLEUM THOMSONI* (Linn)

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As a sequel to a programme of research aimed at the pharmacological evaluation of Indian medicinal plants in this Institute[1], a 50% aqueous EtOH extract of the aerial parts of *H. thomsoni* exhibited spasmolytic and antioxytocic activities in test animals. Subsequent examination of the C_6H_6- (12 g) and EtOAc-soluble (8 g) fractions of the crude extract (169 g from 2 kg dried plant material) where the entire activity was lodged, yielded the following constituents whose characterization and pharmacological evaluation have been described in the present communication.

Chemical investigation: The C_6H_6-soluble fraction on column chromatography over silica gel afforded four constituents, A, B, C and D which were characterized by the study of their spectral data and confirmed by comparing with the respective authentic samples.

The compound A (0.2 g), m.p.138–40°, M^+ m/e 186 $C_{11}H_6O_3$, eluted from 50% C_6H_6 in hexane, was identified as angelicin[2]. Continued elution with the same solvent system afforded B (0.05 g), m.p.161–63°, M^+ m/e 186, $C_{11}H_6O_3$, was identified as psoralen[3]. Compounds C and D were eluted successively from 75% C_6H_6 in hexane. The compound C (0.02 g), m.p.108–9°, M^+ m/e 270, $C_{16}H_{14}O_4$, was characterized as heratomin[4], while the compound D (0.1 g), m.p.189–91°, M^+ m/e 216, $C_{12}H_8O_4$, was identified as sphondin[5].

The EtOAc-soluble fraction similarly yielded additional quantities of A (0.1 g), B (0.03 g), C (0.04 g) and D (0.10 g). Further elution of the column with 2% MeOH in $CHCl_3$ gave bergaptol[6] (0.015 g), m.p. 180–82°, M^+ m/e 202, $C_{11}H_6O_4$.

The *n* BuOH-soluble fraction (1.35 g) of the crude alcoholic extract yielded by droplet counter-current chromatography ($CHCl_3$: MeOH : H_2O, 7 : 13 : 8), apterin[7], m.p. 247–48°, M^+ m/e 424, $C_{20}H_{24}O_{10}$.

Pharmacological study: The plant materials were tested for spasmolytic activity on the isolated guinea pig ileum preparation[8] initially using a concentration of 50 μg/ml against contractions induced by a submaximal concentration of spasmogens, such as ac-

Table 1 *Spasmolytic (guineapig ileum) and antioxytocic (rat uterus) activities*

Substances	Concentration (μg/ml)	Spasmolytic activity % inhibition of			Antioxytocic activity % inhibition of oxytocin
		Acetylcholine	Histamine	$BaCl_2$	
Crude EtOH extract	50	37	41	40	8
	250	—	—	—	31
Hexane fraction	50	40	38	50	10
	250	—	—	—	18
Benzene fraction	10	—	—	50	—
	20	—	—	60	—
	50	87	95	90	70
	250	—	—	—	100
Ethyl acetate fraction	50	53	65	60	16
	250	—	—	—	50
Angelicin	10	—	—	3	—
	20	—	—	5	—
	50	88	95	95	26
	250	—	—	—	100
Psoralen	20	—	—	0	—
	50	62	78	75	11
	250	—	—	—	100
Heratomin	10	—	—	25	—
	20	—	—	54	—
	50	78	92	91	11
	250	—	—	—	80
Sphondin	50	32	48	44	18
	250	—	—	—	33
Bergaptol	10	—	—	22	—
	20	—	—	55	—
	50	72	90	81	71
	250	—	—	—	100

etylcholine (0.01 μg/ml), histamine (0.025 μg/ml) and $BaCl_2$ (20 μg/ml). The spasmolytic activity of the plant materials was assessed by their ability to inhibit the contractions induced by the agonists. The antioxytocic activity was tested on the isolated rat uterus preparation[9] at concentrations of 50 and 250 μg/ml. The activity was assessed by the ability of the test compounds to inhibit the contraction induced by the submaximal concentration of oxytocin (0.1 μ/ml).

The results of the pharmacological activity of the materials obtained from *H. thomsoni* are summarized in table 1. As is evident, the various fractions of the plant showed spasmolytic activity to varying degrees in the isolated guinea pig ileum preparation. The C_6H_6-soluble fraction, however, possessed the maximum activity. At 10 μg/ml concentration it inhibited 50% response of the nonspecific spasmogen, $BaCl_2$. The coumarins (table 1), angelicin, psoralen, heratomin and sphondin isolated from the C_6H_6-soluble fraction and bergaptol from EtOAc-soluble fraction similarly showed spasmolytic activity of almost equal potency indicating that the active principles were the coumarins. All these substances almost equally inhibited the activity of the specific receptor stimulants such as acetylcholine and histamine and also that of the nonspecific spasmogen $BaCl_2$, indicating the nonspecific nature of the spasmolytic activity like papaverine. The fractions as well as the pure coumarins in addition possessed promising antioxytocic activity (table 1), as tested on isolated rat uterus preparation, which is a new finding. Owing to the problems associated with the cultivation of poppy plant, an alternative source of spasmolytic agents are being explored the world over and in this context the coumarins need further exploitation.

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USE OF ORGANIC DEVELOPERS ON DEAE-CELLULOSE FOR THE PURIFICATION OF THE PEPTIDE ANTIBIOTIC MYCOBACILLIN FROM ITS ASSOCIATED PEPTIDE COMYCOBACILLIN

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BUFFERS but not organic solvents are usually employed in biochemical separations by ion-exchange chromatography. However, aqueous organic solvents have sometimes been used as developers in the process[1]. Use of non-aqueous organic solvent mixture has been reported for ion-exchange chromatography of polar lipids only which are insoluble in water[2, 3]. Mycobacillin, an antifungal cyclic tridecapeptide, was isolated as a homogeneous compound in this laboratory[4]. It has recently been observed that mycobacillin prepared now from the culture filtrate of *Bacillus subtilis* B_3 according to the earlier method is not a pure compound but it contains another peptide subsequently named comycobacillin (unpublished). Thus, antibiotic has recently been purified from the mixture[5] by chromatography on DEAE-cellulose using Tris-HCl buffer (10 mM) at pH 7.5. As the method is a little cumbersome, involving further isolation of the antibiotic from the aqueous buffer, a rapid method of purification has been developed and is reported here.

The antibiotic preparation as used for further purification was prepared according to the earlier method[4] from the fermented broth of *B. subtilis* B_3. DEAE-cellulose (medium) was obtained from Sigma Chemical Company, USA and was used as such without any pretreatment. Silica gel G-60 for TLC was obtained from E. Merck, West Germany. Benzene, methanol and glacial acetic acid used were of E. Merck, India and were used as such.

The purity of the antibiotic preparation was tested by TLC on silica gel using *n*-propanol—25% aqueous ammonia (2 : 1 v/v). The spots were located by exposing the plates to iodine vapour[5].

For DEAE-cellulose chromatography, about 200 mg of the cellulose exchanger (hydroxylated form) was suspended in benzene-methanol (90 : 10 v/v), shaken well and then poured into a column having internal diameter 1 cm. The suspension was then allowed to be packed under gravity. In charging the column 10 mg of the antibiotic was dissolved in 0.1 ml methanol and to the solution was added 0.6–0.7 ml benzene (added until the solution became slightly turbid) which was

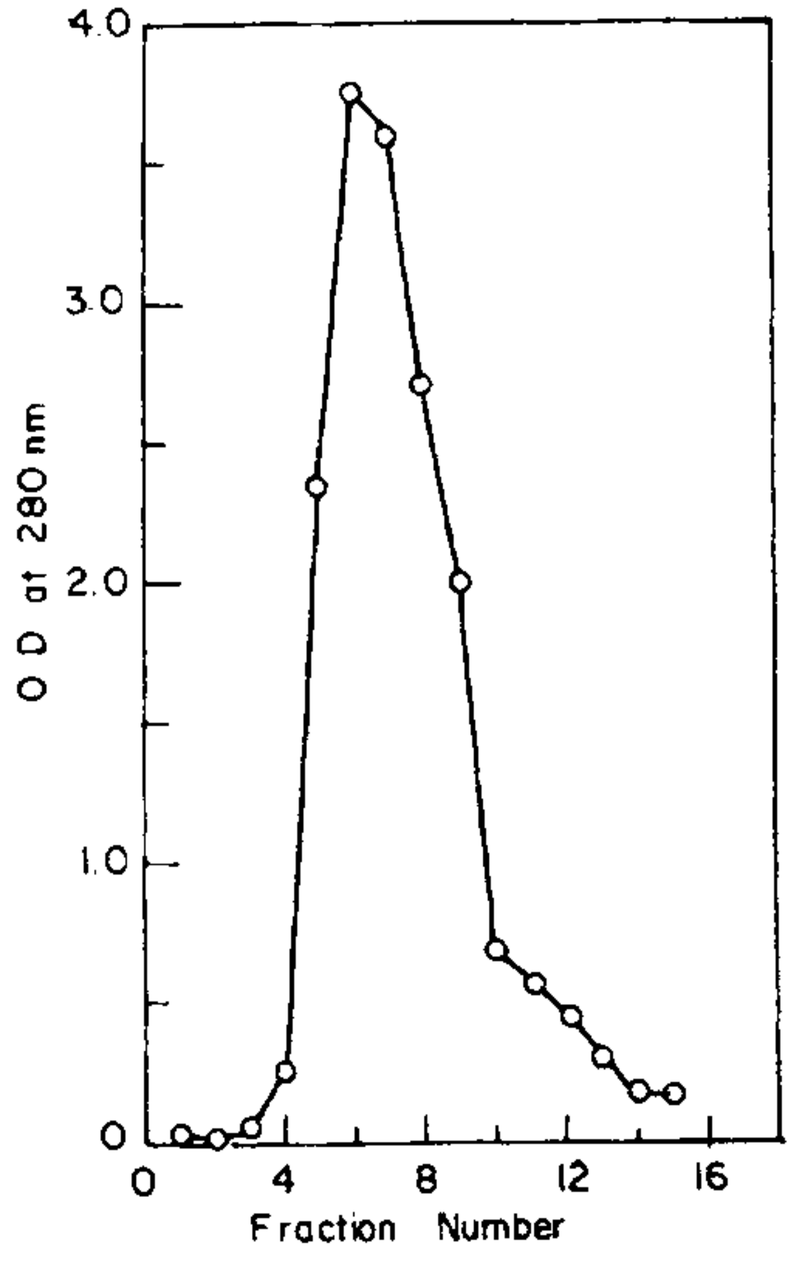

Figure 1. Purification of mycobacillin on DEAE-cellulose column.