Studies on Active Substances in Herbs Used for Hair Treatment. I. Effects of Herb Extracts on Hair Growth and Isolation of an Active Substance from *Polyporus umbellatus* F.¹⁾

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The effects of methanol extracts of 80 herbs on hair growth were investigated, using normal C₃H/He mice from which telogen hair on the back had been removed. Eighteen of the extracts apparently promoted hair regrowth on the mice. As one of active principles in *Polyporus umbellatus* F., 3,4-dihydroxybenzaldehyde was isolated by column chromatography on Amberlite XAD-2, Sephadex LH-20 and silica gel.

Keywords herb; *Polyporus umbellatus*; 3,4-dihydroxybenzaldehyde; C₃H/He mice

Many herbal extracts are used as hair tonics, usually on the basis of long tradition, rather than scientifically proven effectiveness.²⁾ Some of them have been studied in attempts to find a good hair growth promoter,³⁾ and recently lansic acid contained in *Lansium domesticum*, was shown to be effective.⁴⁾

As a part of our studies aimed at identifying active constituents of herbs that promote hair growth,¹⁾ we have examined methanol extracts of 80 herbs, which were selected on the basis of a literature survey.²⁾ Various methods have been reported for evaluating hair growth-promoting ability,^{3,5)} but all of them have disadvantages relating to the species, age, body region or condition of the test animal. In this study, pharmacological hair growth-promoting activity was measured by Ogawa's method using 9-week-old C₃H/He mice,^{5a)} in which the anagen hair regrowth after removal of back telogen hair is evaluated, because this method is considered to be reliable for judging the ability of test samples to promote hair regrowth.

This paper deals with the effects of various herb extracts on hair regrowth and describes the isolation of one of the active principles in *Polyporus umbellatus* F.

Results and Discussion

In relation to hair regrowth, it is well known that 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and all-*trans*-retinoic acid (RA) alter cell proliferation and differentiation in the epithelium and it has also been

suggested that they promote vascular proliferation.^{6,7)} As positive controls for hair regrowth on C₃H/He mice, TPA and RA were used, according to Ogawa and Hattori.^{5a)} On the 8th day after topical application, the regrowth of anagen hair first became morphologically apparent in the control group. At this time, as shown in Table I, the luminance (L) value of the TPA-administered group decreased slightly in comparison with that of the control.¹⁾ TPA showed significant stimulation of hair regrowth on days 10, 13 and 15. RA showed a similar activity to that of TPA, but its effect was more variable. Thus, this method was employed for the first screening of the effective herbs on hair regrowth and for the isolation study on active substances in them.

In this experiment, on days 13 and 15 after TPA application anagen hair regrowth was almost completed and on day 17 hair growth was observed visually. But it was technically difficult to evaluate the effectiveness for hair growth by colorimetry after day 15. Thus, in the following study, measurement was carried out on days 10 and 13, when the condition of regrown hair was suitable for observation.

Accordingly, we examined the effect of methanol extracts of 80 herbs on hair regrowth using this bioassay. As shown in Table II, relative hair growth activity (G.A.) showed that about half of the herb extracts had no apparent effects, while the following results were obtained with the others.

On the 10th day, only 4 of the methanol extracts, from

TABLE I. Effect of TPA and RA on Hair Regrowth in Normal C₃H/He Mice

	Dose (μg/mouse)	0d		3d		6d		8d		10d		13d		15d		17d	
		L	G.A.	L	G.A.	L	G.A.	L	G.A.	L	G.A.	L	G.A.	L	G.A.	L	G.A.
TPA	12.3	54.6 (1.3)	1.3	57.0 (1.3)	−1.8	55.8 (1.1)	0	51.4 (1.5)	3.4	46.7 (1.8) ^{a)}	9.0	36.4 (1.2) ^{a)}	15.2	27.1 (1.4) ^{a)}	20.8	25.2 (0.7)	3.8
RA	4.0	54.8 (1.3)	0.9	56.7 (0.7)	−1.3	55.2 (1.4)	−1.1	51.8 (1.6)	2.6	47.6 (2.6)	7.2	36.3 (6.3)	15.4	26.4 (5.0)	22.8	19.1 (1.3) ^{b)}	27.1
EtOH		55.3 (0.3)	0	56.0 (1.1)	0	55.8 (0.4)	0	53.2 (2.0)	0	51.3 (2.0)	0	42.9 (4.1)	0	34.2 (4.8)	0	26.2 (1.6)	0

L value indicates luminance and each value is the mean (±S.D.). The G.A. value was calculated according to Eq. 1. a) and b) indicate significant differences from the control (EtOH), $p < 0.05$ and $p < 0.01$, respectively.

TABLE II. Effect of Herb Extracts and Catechol on Hair Regrowth in Normal C₃H/He Mice^{a)}

Herb	Family	Yield (%)	n	10 d	13 d	Herb	Family	Yield (%)	n	10 d	13 d
				G.A.	G.A.					G.A.	G.A.
<i>Crataegus cuneata</i> S. et Z.	Rosaceae	10.5	4	7.9 ^{c)}	19.7 ^{c)}	<i>Phytolacca esculenta</i> van H.	Phytolaccaceae	9.5	4	-1.1	0
<i>Ligustrum lucidum</i> A.	Oleaceae	28.0	4	7.0 ^{c)}	15.7 ^{c)}	<i>Juncus decipiens</i> N.	Juncaceae	7.5	4	-0.2	0
<i>Polyporus umbellatus</i> F.	Polyporaceae	1.0	4	8.3 ^{b)}	14.3 ^{b)}	<i>Loranthus parasiticus</i> (L.) M.	Loranthaceae	11.0	4	1.6	0
<i>Brassica hirta</i> M.	Cruciferae	27.0	5	2.7	13.2 ^{b)}	<i>Ligusticum sinense</i> O.	Apiaceae	16.0	4	1.0	-0.2
<i>Vitex rotundifolia</i> L. fil.	Vervaceae	10.5	5	2.1	13.2	<i>Prunus ansu</i> K.	Rosaceae	29.5	4	-1.5	-0.3
<i>Pachyma hoelen</i> R.	Polyporaceae	2.0	4	3.8	11.6	<i>Sanguisorba officinalis</i> L. var. <i>carnea</i> R.	Rosaceae	24.5	4	0	-0.4
<i>Bletilla striata</i> (T.) R. fil.	Orchidaceae	5.5	4	4.5 ^{b)}	10.9 ^{b)}	<i>Pharbitis nil</i> (L.) C.	Convolvulaceae	21.0	4	0.2	-0.7
<i>Polygala senega</i> L.	Polygalaceae	24.8	4	2.9	9.0	<i>Asiasarum sieboldii</i> (M.) F. M.	Asaraceae	11.7	5	0.2	-0.9
var. <i>latifolia</i> T. et G.						<i>Punica granatum</i> L.	Punicaceae	32.0	4	-3.8 ^{b)}	-1.0
<i>Magnolia kobus</i> DC.	Magnoliaceae	13.8	5	-0.2	7.6	<i>Prunus japonica</i> T.	Rosaceae	33.5	4	0.2	-1.2
<i>Dictamnus albus</i> L.	Rutaceae	4.0	4	2.3	7.0	<i>Rosa polyantha</i> S. et Z. var. <i>genuina</i> N.	Rosaceae	21.0	4	-3.3	-1.4
subsp. <i>dasycarpus</i> (T.) K.						<i>Zea mays</i> L.	Gramineae	3.0	4	-1.1	-1.5
<i>Valeriana officinalis</i> L.	Valerianaceae	11.5	4	2.8	6.6	<i>Cocculus trilobus</i> A. P. DC.	Stephania	3.0	3	-0.4	-1.6
var. <i>latifolia</i> M.						<i>Alisma orientale</i> J.	Alismataceae	13.0	3	0	-1.7
<i>Phyllostachys nigra</i> M.	Bambusaceae	16.0	4	5.4	6.0	<i>Selinum monniieri</i> L.	Apiaceae	9.0	4	-0.2	-2.2
var. <i>henosis</i> S.						<i>Pinus densiflora</i> S. et Z.	Pinaceae	25.5	4	1.1	-2.4
<i>Ampelopsis japonica</i> M.	Vitaceae	3.5	4	2.9	6.0	<i>Terminalia chebula</i> R.	Combretaceae	24.0	4	-1.9	-2.9
<i>Daphne genkwa</i> S. et Z.	Thymelaeaceae	8.5	4	2.8	6.0	<i>Juglans regia</i> L. var. <i>sinensis</i> DC.	Juglandaceae	9.5	4	-2.4	-3.6
<i>Artemisia gigantea</i> K.	Carduaceae	10.0	3	2.1	5.9	<i>Curcuma longa</i> L.	Zingiberaceae	15.0	4	0.2	-3.7
<i>Glehnia littoralis</i> F. S.	Glehnia	6.5	5	3.5	5.6	<i>Lepidium apetalum</i> W.	Cruciferae	12.0	4	-2.0	-3.9
<i>Leonurus heterophyllus</i> S.	Labiatae	2.0	4	2.0	5.3	<i>Sophora angustifolia</i> S. et Z.	Leguminosae	13.5	4	-1.9	-3.9
<i>Benincasa cerifera</i> S.	Cucurbitaceae	8.5	4	-0.2	5.3	<i>Dipsacus asper</i> W.	Dipsacaceae	27.5	4	-1.7	-4.2
<i>Pulsatilla cernua</i> (T.) S.	Ranunculaceae	11.0	4	1.7	4.8	<i>Coptis japonica</i> M.	Ranunculaceae	13.0	4	-0.7	-4.6
<i>Asparagus cochinchinensis</i> M.	Liliaceae	15.0	4	2.8	4.6	<i>Cassia tora</i> L.	Cassiacae	12.7	5	-3.1	-4.6
<i>Laminaria japonica</i> A.	Laminariaceae	12.5	4	3.3	4.2	<i>Scutellaria baicalensis</i> G.	Labiatae	14.0	4	-0.5	-4.8
<i>Akebia quinata</i> (T.) D.	Lardizabalaceae	5.0	3	0.9	4.1	<i>Cnidium officinale</i> M.	Umbelliferae	14.0	4	-1.1	-5.0
<i>Aquillaria sinensis</i> (L.) G.	Thymelaeaceae	34.5	4	2.3	3.9	<i>Psoralea corylifolia</i> L.	Leguminosae	27.5	4	-1.9	-5.5
<i>Plantago major</i> L. var. <i>asiatica</i> D.	Plantaginaceae	9.0	4	0.7	2.9	<i>Rhus javanica</i> L.	Anacardiaceae	75.0	4	-0.6	-5.8
<i>Nardostachys jatamansi</i> DC.	Valeriaceae	21.5	4	-0.5	2.9	<i>Broussonetia papyrifera</i> (L.) V.	Moraceae	14.0	4	-1.6	-7.3
<i>Dianthus superbus</i> L.	Caryophyllaceae	10.0	4	-1.3	2.4	<i>Aconitum japonicum</i> T. var. <i>genuinum</i> N.	Ranunculaceae	8.5	4	-3.1	-7.7
<i>Paeonia lactiflora</i> P.	Ranunculaceae	36.5	3	1.1	2.4	<i>Rehmannia glutinosa</i> L.	Scrophulariaceae	52.5	4	-2.8	-8.7
<i>Curcuma zedoaria</i> R.	Zingiberaceae	4.5	4	-0.6	2.1	<i>Crocus sativus</i> L.	Iridaceae	56.0	3	-2.7	-10.4
<i>Dioscorea tokoro</i> M.	Dioscoreaceae	6.0	4	1.4	1.7	<i>Agastache rugosa</i> O. K.	Lamiaceae	11.0	4	-3.4 ^{b)}	-11.6 ^{b)}
<i>Cannabis sativa</i> L.	Cannabinaceae	11.0	4	-0.2	1.6	<i>Biota orientalis</i> E.	Cupressaceae	14.0	4	-6.0	-11.9
<i>Coix lachryma-Jobi</i> L.	Gramineae	7.5	4	-1.6	1.5	<i>Boswellia carterii</i> B.	Burseraceae	53.5	4	-5.4	-12.7 ^{b)}
<i>Eclipta prostrata</i> L.	Carduaceae	4.5	4	2.0	1.5	<i>Cimicifuga foetida</i> L.	Ranunculaceae	8.0	4	-3.9	-13.2
<i>Ligusticum acutilobum</i> S. et Z.	Umbelliferae	22.9	5	-2.7	1.2	<i>Santalum album</i> L.	Santalaceae	3.5	4	-4.3	-13.3
<i>Angelica dahurica</i> var. <i>pai-chi</i> K. H. et Y.	Apiaceae	21.5	3	0.4	1.1	<i>Chrysanthemum morifolium</i> R.	Compositae	17.5	4	-3.8	-14.8
<i>Polygonum aviculare</i> L.	Polygonaceae	8.5	4	1.6	1.0	<i>Rheum palmatum</i> L. var. <i>tanguticum</i> M.	Polygonaceae	23.0	4	-4.0	-15.0 ^{b)}
<i>Gardenia jasminoides</i> E. var. <i>grandiflora</i> N.	Rubiaceae	21.0	4	0.7	0.7	<i>Zingiber officinale</i> R.	Zingiberaceae	9.5	4	-4.6 ^{c)}	-16.9 ^{c)}
<i>Citrus aurantium</i> L.	Rutaceae	28.6	5	-1.3	0.6	<i>Abutilon avicennae</i> G.	Malvaceae	13.5	4	-9.0 ^{b)}	-17.2
<i>Cassia angustifolia</i> V.	Leguminosae	18.0	4	-3.1	0.5						
<i>Kochia scoparia</i> (L.) S.	Chenopodiaceae	6.0	4	-1.1	0.2						
						Catechol					27.2 ^{c)}

The G.A. value was calculated according to Eq. 1. a) The doses of herb extract and catechol were 2 mg/mouse and 1.1 mg/mouse, respectively. b) and c) indicate significant differences from the control, $p < 0.05$ and $p < 0.01$, respectively.

Crataegus cuneata S. et Z., *Ligustrum lucidum* A., *Polyporus umbellatus* F. and *Bletilla striata* (T.) R. fil., significantly promoted hair regrowth. On the 13th day, 18 kinds of herb extracts showed apparent hair regrowth promotion (G.A. > 5.0) visually. In particular, the extracts of the 4 herbs mentioned above and *Brassica hirta* M. showed significant activities almost equal to those of the positive controls, TPA and RA. There have been previous reports on 4 herbs, *Magnolia salicifolia* M., *Vitex rotundifolia* L. fil., *Brassica hirta* M. and *Dictamnus albus* L. subsp. *dasycarpus* (T.) K., of the 18 which stimulated hair growth.^{3a,b)} There is no apparent relation between their families and activities.

On the other hand, clear inhibition of hair regrowth was observed with 16 kinds of herb extracts (G.A. < -5.0), among which the extracts of *Abutilon avicennae* G., *Zingiber officinale* R., *Rheum palmatum* L. var. *tanguticum* M., *Boswellia carterii* B. and *Agastache rugosa* O. K.,

TABLE III. Materials with Potent Hair Regrowth-Promoting Activity in Normal Mice

Name	Part
<i>Crataegus cuneata</i> S. et Z. (Sanzashi)	Fruit
<i>Ligustrum lucidum</i> A. (Joteishi)	Fruit
<i>Polyporus umbellatus</i> F. (Chyorei)	Fungal body
<i>Brassica hirta</i> M. (Hakugaishi)	Seed
<i>Vitex rotundifolia</i> L. fil. (Mankeishi)	Fruit
<i>Pachyma hoelen</i> R. (Bukuryou)	Fungal body
<i>Bletilla striata</i> (T.) R. fil. (Byakkyuu)	Tuber

showed significant effects. It has been reported that *Zingiber officinale* R. inhibited hair growth in mice.^{3a)} The inhibitions by *Rheum palmatum* L. var. *tanguticum* M. and *Boswellia carterii* B. may be derived from their anti-inflammatory effects, as betamethasone valerate completely inhibits hair regrowth in C₃H/He mice.^{5a)}

Chart 1. Isolation of Active Constituents from *Polyporus umbellatus* F.

() indicates yield. [,] indicates daily application dose (/mouse) and time (d), and the average hair growth score.

TABLE IV. Relationship between G.A. and Scoring of Hair Regrowth of Mice after Removal of Normal Hair

G.A.	—5.0	—4.9—1.0	—0.9—3.0	3.1—10.0	10.1—16.0	16.1—21.0	21.1—25.0	25.1—29.0	29.1—
Score	—2	—1	0	1	2	3	4	5	6

The G.A. value was calculated according to Eq. 1.

As shown in Table III, about half of the 7 highly potent herbs with G.A. values over 10.0 were fruits or seed, which contain relatively high contents of fatty acids. It is known that linsic acid and pentadecanoic acid triglyceride promote hair growth.^{4,8)} Speculating that lipophilic acids in those herbs might be active components, we have studied the active principle in *Vitex rotundifolia* L. fil. and found that a mixture of fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid and eicosanoic acid) showed the hair regrowth activity.⁹⁾ Studies on the other fruits or seed are in progress.

We were particularly interested in *Polyporus umbellatus* F. and *Pachyma hoelen* R. among the 7 active herbs, as the contents of fatty acids in the fungal bodies are very low, and there has been no previous report on their hair regrowth-promoting activities. We have therefore studied the active components in *Polyporus umbellatus* F.

Bioassay-guided fractionation of *Polyporus umbellatus* F., as shown in Chart 1, afforded 3,4-dihydroxybenzaldehyde as an active compound after 3 steps of column chromatography on Amberlite XAD-2, Sephadex LH-20 and silica gel. The activity during purification is presented in Chart 1 as the average scores for simplicity, according to the hair regrowth scoring system shown in Table IV.

As shown in the chart, ground fungal body of *Polyporus umbellatus* F. was extracted with ethanol and water (1 : 1) under reflux. The extract, which showed potent activity,

was dissolved in the same solvent at 10°C, and subjected to Amberlite XAD-2 column chromatography using methanol and water as an eluent. The activity emerged in all fractions. In this paper, we will deal with fraction I₂. Column chromatography of active fraction I₂ on Sephadex LH-20 gave five fractions, II₁ to II₅, using MeOH–H₂O as an eluate. The most potent activity was found in fraction II₄. The final purification of the active compound in fraction II₄ was achieved by silica gel column chromatography. Recrystallization of the product III₁ eluted with CHCl₃–MeOH (9 : 1) from H₂O afforded the compound as colorless plates, which showed activity. Work on the constituents of the other active fractions is in progress.

The spectral (IR, UV, NMR and MS) data of the isolated active principle suggested it to be 3,4-dihydroxybenzaldehyde. This conclusion was confirmed by direct comparison of the spectral data with those of authentic 3,4-dihydroxybenzaldehyde obtained from Wako Co. The activity of the authentic sample was equal to that of the isolated compound, as shown in Table V.

3,4-Dihydroxybenzaldehyde is known to be a component of *Pinellia ternata*.¹⁰⁾ It has anti-arthritis and anti-inflammatory activities,¹¹⁾ and inhibits cancer cell proliferation,¹²⁾ but this is the first report that the compound also promotes hair regrowth. It is of interest to consider the relationship between the chemical structure and biological activity of 3,4-dihydroxybenzaldehyde and catechol. 3,4-Dihydroxybenzaldehyde is fairly stable in

TABLE V. Effect of 3,4-Dihydroxybenzaldehyde on Hair Regrowth in Normal C₃H/He Mice

Dose (mg) ^{a)}	0.5	1.0	2.0
G.A.	0.5	0.7	5.4

a) It indicates daily application dose (/mouse) 4 times (d). The G.A. value was calculated according to Eq. 1 ($n=7$ or 8).

aqueous solution and has anti-inflammatory activity, but catechol is very unstable and causes irritation,¹³⁾ although it has potent hair regrowth activity, as shown in Table II. The fact that both compounds promote hair regrowth, suggests that the catechol moiety is the key structure for inducing the cell proliferation and differentiation required for hair regrowth.^{12,14)}

In conclusion, it was found that Ogawa's method using the back hair of C₃H/He mice is effective for evaluating pharmacological activity of test materials to promote hair regrowth, and several herb extracts were found to promote hair regrowth significantly. In view of the stability and anti-inflammatory activity of 3,4-dihydroxybenzaldehyde, an active ingredient isolated from *Polyporus umbellatus* F., it appears to be a good candidate for use in hair growth preparations. Studies on the mechanism of hair growth promotion by 3,4-dihydroxybenzaldehyde are under way.

Experimental

Hair Regrowth-Promoting Activity of 80 Herb Extracts Materials: Commercial herb products were used in this study. TPA and RA were purchased from Wako Co.

Preparation of Test Samples: Samples of pulverized herbs (20 g) were extracted with MeOH (200 ml) under reflux for 2 h. Each extract was evaporated *in vacuo*, and 2 mg of the residue was dissolved in 20 μ l of EtOH for use as a test sample. The yields (%) of the extracts are shown in Table I. TPA and RA were dissolved in EtOH at concentrations of 1.0 mM and 0.02% (w/v), respectively. RA was used together with vitamin E (1.0% (w/v)) according to Ogawa and Hattori.^{5a)} EtOH was used as the control.

Measurement of Hair Regrowth Activity in Mice: The colorimeter used for the measurement of luminance was a Minolta Chroma meter CR-100 (Minolta Camera Co.) or a DS-506 (Keihin Densoku Co.).

Four 9-week-old male C₃H/He mice (age at the initial application of test material) were used per group in general. The hair on the back was clipped off with hair clippers (*ca.* 3 $\times$ 4 cm²). The clipped area was treated with softening cream (Jully, Kowa Co. or Simfree for etiquette, Pola Co.) for 5 or 10 min then the cream was washed off with warm water. After 24 h, the test sample was applied topically once. The luminance (L) of the depilated area was measured on various days (shown in Tables I and II) after application with a colorimeter and the G.A. was calculated by use of the averaged luminance (L) value of each group, according to the following equation. Statistical significance of differences between groups was assessed by using Student's *t* test.

$$\text{G.A. (\%)} = \left(1 - \frac{\text{mean luminance of administered group}}{\text{mean luminance of control group}} \right) \times 100 \quad (1)$$

Isolation of Active Principle from *Polyporus umbellatus* F. Instruments Used Were as Follows: mass spectrum (MS), JEOL DX-300 spectrometer; infrared (IR) spectrum, JASCO IR-700 spectrophotometer; nuclear magnetic resonance (NMR) spectra, JEOL GSX-270 and JEOL GSX-500 Fourier-transform spectrometer; UV spectrum, Hitachi model 200-20 spectrophotometer.

Measurement of Hair Regrowth Activity of Each Fraction in Mice: The method was as described above, with 8 or 9 animals per group. Test material was dissolved in 30 μ l of EtOH-H₂O (1:1), and applied once a day for 4 d. EtOH-H₂O (1:1) was used as the control. The effect of the material on hair regrowth was scored (see Table IV) on the 13th day after the first application.

Extraction: Ground *Polyporus umbellatus* F. (9.6 kg) was extracted with EtOH-H₂O (1:1, 96 l) under reflux for 3 h. The solution was filtered,

and the filtrate was evaporated *in vacuo* to give the crude extract (76.8 g). The extract was exposed to EtOH-H₂O (1:1, 920 ml) at 10 °C for 14 d. The soluble part was concentrated in an evaporator to afford the extract (35.12 g).

Amberlite XAD-2 Column Chromatography of the Extract: The extract (35.12 g) was dissolved in H₂O (1 l), and applied to an Amberlite XAD-2 column (5 $\times$ 60 cm). Elution with water (2.5 l), MeOH-H₂O (1:1, 6 and 9 l) and MeOH (5 l) afforded fractions I₁ (23.46 g) (water effluent), I₂ (5.08 g) (MeOH-H₂O), I₃ (0.42 g, MeOH-H₂O) and I₄ (1.87 g, MeOH).

Chromatography of Fraction I₂ on Sephadex LH-20: Fraction I₂ (5.08 g) was subjected to gel filtration on a Sephadex LH-20 column (980 ml, 4.5 $\times$ 62 cm). Elution with MeOH-H₂O (1:1) gave Fraction II₁ (3.64 g), II₂ (0.59 g), II₃ (0.42 g), II₄ (0.22 g) and II₅ (0.12 g) (K_{av} value = 0 to 0.65, 0.65 to 1.14, 1.14 to 2.24, 2.24 to 2.89 and 2.89 to 5.00, respectively).

Silica Gel Column Chromatography of II₄: Fraction II₄ (0.22 g) was subjected to column chromatography on a silica gel column (1.5 $\times$ 33 cm). Elution with CHCl₃-MeOH (9:1, 100 ml), CHCl₃-MeOH (1:1, 100 ml), MeOH (100 ml) and AcOH-H₂O (0.5:99.5, 100 ml) afforded III₁ (156.3 mg) (CHCl₃-MeOH (9:1)), III₂ (17.7 mg) (CHCl₃-MeOH (1:1)) and III₃ (45.0 mg) (MeOH and AcOH-H₂O). Recrystallization of III₁ from H₂O gave colorless plates (123.4 mg) of the active compound.

Identification of the Active Compound: The active compound (dec. 152–153 °C (dec. 153–154 °C))¹⁵⁾ was identified as 3,4-dihydroxybenzaldehyde by direct comparison of its physical data (IR, UV, MS and NMR) with those of an authentic sample.

References and Notes

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