4.8 to 8 mg which was varied from 50 to 80% of the loading dose per sq cm of the pseudolatex patch.

The various physicochemical properties of the pseudolatex films, such as thickness, weight variation, tensile stength, percentage of elongation, uniformity of drug content and moisture absorption properties were found to be within limits and satisfactory. The skin irritation test conducted over rabbits showed that there was no irritation at all for a period of 7 d, establishing the superiority of pseudolatex patches over other types of transdermal films. The *in vitro* release profile and skin permeation studies showed that the pseudolatex patches could be used as suitable sustained released transdermal drug delivery system over a prolong period of time.

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Novel Method of Synthesis of Curcuminoids and Derivative of Ibuprofen as Potent Antiinflammatory Agent

P. JAYASEKHAR*, S. B. RAO AND G. SANTHAKUMARI

Priyadarshini Institute of Paramedical Sciences, Medical College, Thiruvananthapuram-695 011

*Department of Pharmacy, T. D. Medical College, Alappuzha-688 005.

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A novel method of synthesis of curcumin-I has been successfully developed using Claisen-Dieckmann condensation where dehydrozingerone is condensed with methyl ferulate in presence of sodium ethoxide in dimethyl sulphoxide. Various curcuminoids are prepared by changing the esters and α, β-unsaturated ketones. This reaction is utilized to prepare dehydrozingerone-

*For correspondence

E-mail: jaygeeth@eth.net.in

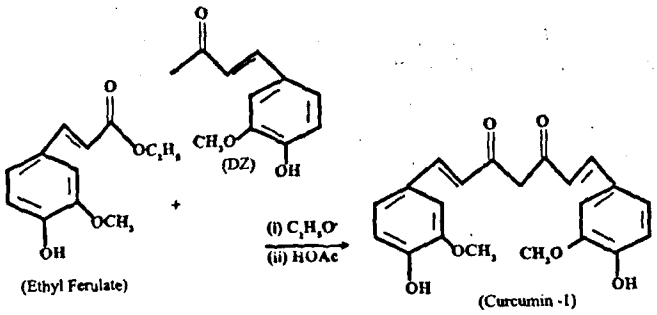
ibuprofen condensation product (DZIBU), a potent antiinflammatory agent. The homogenecity of compounds synthesized was established and structures were confirmed by spectral data. DZIBU exhibited powerful antiinflammatory activity on carrageenan-induced paw edema model and formaldehyde-induced arthritis model in rats. Though the analgesic activity was less when compared to that of ibuprofen, the new compound did not induce gastrointestinal side effect in animal studies.

Curcuminoids refer to a group of phenolic pigments present in the dried rhizomes of *Curcuma longa*[1]. They can be considered as dimers of dehydrozingerone and are chemically diferulmethane derivatives. They are reported to be powerful antioxidants with anti-inflammatory, anticancer and antimutagenic properties. Curcumin, like aspirin has been reported to inhibit cyclooxygenase activity of platelets and reduce platelet thromboxane B2 levels[2]. The major constraints of curcumin in the use as therapeutic agent is its high cost and poor gastrointestinal absorption. Economical synthesis and structural modification were attempted to produce potent analogues. Babu and Rajasekharan modified Pabon's synthesis of curcumin by reacting the vanillin and acetylacetone in dimethyl formamide (DMF) in presence of boric acid and 1,2,3,4,-tetrahydroquinoline[3]. This method has a disadvantage as bis derivatives having identical 1,7-sybstitutions on 1,6-heptadiene-3, 5-dione is only possible.

A new method for the synthesis of curcumin I has been developed by us using Claisen-Dieckmann condensation[4], where dehydrozingerone is condensed with methyl ferulate in presence of sodium ethoxide in dimethyl sulfoxide. Dehydrozingerone is reported to exhibit mild analgesic and antiinflammatory activities. Chemically it is one half of the molecule of curumin-I. In order to develop novel therapeutic agent with enhanced antiarthritic and antiinflammatory activities dehyrozingeone and methyl ester of ibuprofen are combined by Claisen-Dieckmann condensation reaction to give *(E)-6-(p*-isobutylphenyl)-1-(4'-hydroxy-3'-methoxyphenyl)-1-hepten-3, 5-dione. The percentage yield of curcumin-I synthesized by this new method is similar to that one reported by Babu and Rajasekharan[3]. Demethoxycurcumin (DMC), bis-demethoxycurcumin (BDMC), curcumin-II methyl ether and curcumin-III demethyl ether were also synthesized in the same manner utilizing suitable esters and α, β-unsaturated ketones.

Melting points were determined on electrothermal apparatus in open capillary tubes. The IR spectra were recorded on Perkin Elmer model 852 IR spectrometer. The electronic absorption spectra were recorded on Shimadzu GBC double beem UV-Vis spectrophotometer. The ^{1}H NMR spectra were recorded on a Joel (EM-390, 90 MHz) NMR spectrometer, using tetramethylsilane (TMS) as internal standard (chemical shifts expressed in ppm), of photochemistry division of Regional Research Laboratory, Trivandrum. The mass spectra were recorded on a Finnigan Mat 8230 Mass spectrometer using Maspec system MSW/9629 series II Gas Chromatography set up (25 m lenth, 0.2 mm ID, capillary column and FID detector) at Regional Sophisticated Instrumentation Center, IIT, Chennai. Elemental analyses were performed in Perkin Elmer 2400 Elemental Analyser.

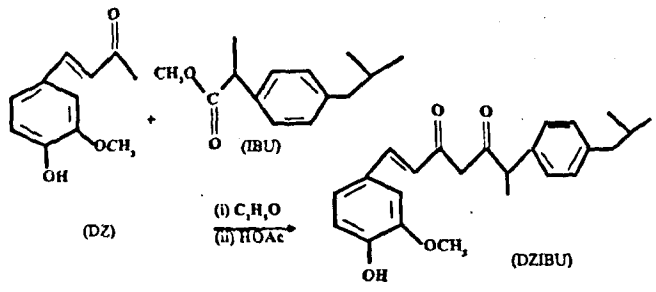
Dehydrozingerone was synthesized by modified aldol condensation namely Claisen-Schmidt reaction where vanillin is condensed with acetone in presence of aqueous sodium hydroxide and followed by acidification with dilute acetic acid[5]. Curcumin was synthesized by Claisen-Dieckmann condensation. Finely powdered clean sodium (0.92 g, 0.04 mol) and 25 ml dry xylene was transferred to a 250 ml three necked flask. The xylene was decanted as completely as possible and washed with dry solvent ether in 10 ml portions. Twenty milliliters solvent ether was added, the flask was placed on a water bath and was fitted with a dropping funnel, Hershberg sealed stirrer and a reflux condenser. Guard tubes were inserted containing absorbent cotton and anhydrous calcium chloride. Ethanol (2.5 g, 0.05 mol) was added from the dropping funnel with gentle reflux, and stirring continued till all sodium was reacted (about 1 h). The ether was distilled as completely as possible and DMSO (10 ml) was added to the warm sodium ethoxide. After cooling to room temperature, dehydrozingerone (1.92 g, 0.01 mol) dissolved in 10 ml DMSO was added. Ethyl ester of ferulic acid (2.2 g, 0.01 mol) in DMSO (10 ml) was added in small quantity with stirring over a period of 1 h, the reactants in the flask were heated at 60° on a water bath for 1 h, and transferred to a beaker containing 100 ml crushed ice. The solution was acidified with dilute acetic acid and the solid separated out was washed with 5% sodium bicarbonate solution. The crude product obtained was purified by dry column chromatography using silica gel (120-



Synthesis of Curcumin - 1 (Scheme - 1).

200 mesh) and eluted with chloroform. The solvent was remove to afford the product (Scheme I). Yield: 2.6 g (76%); bright orange yellow crystals. m p. 180-82° (Lit 183°). UV λ max (CH_3OH): 242, 418 nm; IR vmax (KBr): 3545, 1640, 1612, 1515, 1440, 1370, 1290, 1242, 1220, 1200, 1168, 1035, 975, 870, 825, 788 cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz): δ 1.30 (1H, s, H-4), 3.34 (6H, s, 2x – OCH3), 6.50 (2H, m, H-2,6), 6.94 (2H, d, J=16Hz, H-1, 7), 6.98 (1H, m, H-4), 7.15 (2H, d, J=4 Hz and 8Hz, H6-Ar), 7.3 (2H, d, J=2 Hz, H2-Ar), 7.68 (2H, d, J=2Hz, 2H-Ar). Mass spectrum m/z: 368 (M^+, 10), 337 (10), 313 (5), 294 (10), 258 (15), 238 (15), 206 (30), 178 (100), 161 (100), 135 (55), 89 (40), 77 (60). Anal. Calcd for ($C_{21}H_{20}O_6$): C, 68.48; H, 5.43, Found: C, 68.12; H, 5.20.

Similarly other curcuminoids derivatives were prepared by condensing suitable α, β-unsaturated ketones. And ester of α,β-unsaturated carboxylic acid under same condition as described for the synthesis of curcumin-I. Similar procedure and conditions for the synthesis of curcumin-I was adopted to prepare ibuprofen derivative, except the replacement of ethyl ester of ferulic acid with methyl ester of ibuprofen (2.2 g, 0.01 mol). The crude product separated upon acidification with acetic acid was recrystallized from methanol to give pale yellow needles (Scheme 2). Yield: 2.81 g (74%) m.p 238-39°. UV λmax (CH_3OH): 232, 287 nm;

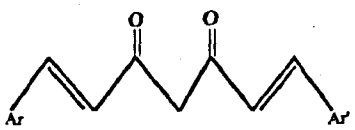


Synthesis of DZIBU - 1 (Scheme - 2).

IR vmax (KBr): 3429, 1920, 1719, 1610, 1521, 1465, 1423, 1328, 1230, 1179, 1070, 1910, 935, 864, 778 cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz) δ 0.089 (6H,d,J=6 Hz (CH_3)$_2$), 1.51 (3 H, d, J=8 Hz, CH_3CH), 1,78 (1 H, m, CH (CH_3)$_2$), 2.40 (2H, d, J=6 Hz, CH_2-Ar), 2.35 (3H, s, COH_3), 3.63 (2H, s, O=C-CH_2-C=O), 3.79 (1H, q, CH_3CH), 3.85 (3H, s, -OCH_3), 6.74 (2H, each d, J=12Hz *(E)*-CH= CH-), 6.85 (1H, s, H6-Ar), 7.8 (4H, m, H2,3,5,6-Ar): Mass spectrum m/z : 380 (M^+, 10), 286 (20), 214 (10), 206 (50), 161 (100), 150 (20), 135 (50), 119 (60), 91 (80), 77 (20). Anal. Calcd for ($C_{24}H_{28}O_4$): C,75.79; H,7.37, Found: C, 75.23; H, 7.14.

Oral toxicity (LD_{50}) of the compound DZIBU, was determined in albino mice in groups of 6 of either sex for each dose tested. One tenth to one fifteenth of oral LD_{50} was selected as an oral dose for the pharmacological testing. Fine suspension of the test compounds in 1% sodium carboxymethylcellulose was employed for biological testing and doses were expressed in m mol/kg, in order to compare the pharmacological activities with parent compound[6]. Carrageenan-induced rat paw edema (acute antiinflammatory: non immuno model) was performed by the technique of Winter *et al*[7]. The edema was induced in albino rats in groups of 6 by injecting 0.1 ml carrageenan (1% w/v suspension in normal saline) into sub planter region of the left hind paw. The volume of paw was measured immediately using a plethysmometer after

TABLE 1: CHEMISTRY OF CURCUMINOIDS SYNTHESIZED.



Compounds	1-Ar	7-Ar'	%yield	m.p°
Curcumin I	3-methoxy-4-hydroxy	3-methoxy-4-hydroxy	54	180-82
Curcumin II (DMC)	3-methoxy-4-hydroxy	4-hydroxy	61	185-88
Curcumin II methyl ether	3-methoxy-4-hydroxy	4-hydroxy	53	178-81
Curcumin II dimethyl ether	4-methoxy	4-methoxy	60	165-68

Melting points were determined in open capillary tybes and were uncorrected.

TABLE 2: ANALGESIC AND ANTIINFLAMMATORY ACTIVITY STUDIES OF DZIBU

Compd	Oral dose (mmol/kg)	Analgesic Activity@		Antiinflammatory Activity# Carrageenan-induced rat paw edema method			
		Writhing in 20 min.@	% Activity	Normal rats # (Group I)		Adrenalectomised rats $ (Group II)	
				Mean increase in edema (ml)#	% Activity	Mean increase in edema (ml)$	% Activity
Control	-	65.8±4.76	0	0.70±0.039	0	0.65±0.041	0
DZIBU	0.375	48.7±6.50*	26	0.40±0.024*	43	-	-
DZIBU	0.500	35.6±3.10*	45	0.34±0.022*	52	0.39±0.032*	40
DZIBU	0.625	32.2±3.22*	51	0.25±0.020*	64	-	-
DZIBU	0.750	29.5±2.25*	55	0.20±0.018*	71	-	-
DZIBU	1.000	26.8±2.64*	59	0.18±0.019*	74	0.20±0.180*	70
IBU	0.500	30.5±2.25*	53	0.42±0.032*	40	0.39±0.026*	40
IBU	1.000	20.4±20.86*	69	0.21±0.028*	70	0.19±0.020*	71

@ Effect of test compound (DZIBU) and the standard, ibuprofen (IBU) on acetic acid-induced writhing syndrome in albino mice. # Effect of test compound on carrageenan-induced paw edema in normal rats; $ the same in adrenalectomised rats. The values are mean±S.E (n=6) *P<0.05, significantly different when compared with the control group (Student's t test). In # and $ the values are mean±S.E (n=6) not significantly different when Group I and Group II are compared (P>0.5).

carrageenan injection and again 3 h later. The test compounds (0.5 and 1 mmol/kg b.w) were given orally 1 h prior to carrageenan injection. Mean increase in paw volume and standard error (S.E.) were calculated, and results were expressed as % inhibition of edema as compared to the control. Ibuprofen was used as standard drug. The test was also repeated in adrenalectomised rats by the method of Schultzer[8]. The antiarthritic activity of test compound (0.5 and 1 mmol/kg) were carried out in adult male rats using formaldehyde-induced arthritis model of Brownlee[9]. Formaldehyde solution was injected subcutaneaously under planter aponeurosis in the left hind paw on the first and third days. Test compounds (0.5 mmol/kg) were given orally daily for 10 d. Day to day changes in the inflammatory reactions were assessed by measuring the volume of paw using plethysmometer and results were statistically evaluated.

The effect of test compound (0.5 mmol/kg) in chronic inflammation and granuloma formation was tested on albino rats of either sex using the method of Winter and Porter[10]. Cotton pellets were implanted subcutaneously one each in both the auxillae and groin region in animals under light ether anaesthesia. The test compounds were given orally every day for seven days starting from the day of pellet implantation. The control had vehicle and positive control was ibuprofen (0.5 mmol/kg). On day 8, they were sacrificed, pellets were removed, cleaned from extraneous tissue and dried at 60° overnight. The pellets were weighed and the net weight expressed as average weight of animals treated as a group. The percentage inhibition was calculated taking the weight gain in control as 100%.

Analgesic activity of the test compounds were determined by acetic acid-induced writhing syndrome method of Taber *et al*[11]. The test compounds were administered orally 30 min prior to acetic acid injection. The mice were placed individually into glass beakers and 5 min were allowed to elapse and then observed for period of 20 min. Number of writhing was recorded for each animal and the analgesic activity was determined as percentage reduction of writhing in test animal in comparison with that of control. Ibuprofen in same dose level was used as positive control. The ulcerogenic effect was determined using the method described by Cashin *et al*[12]. The severity of gastric lesion appearing in the glandular or muscular portions were

TABLE 3: EFFECT OF TEST COMPOUNDS ON FORMALDEHYDE-INDUCED ARTHRITIS IN ALBINO RATS.

Compound	Mean rat paw edema (ml)±S.E during treatment (d)								
	2	3	4	5	6	7	8	9	10
Control	0.42± 0.050	0.61± 0.048	0.84± 0.042	0.73± 0.041	0.74± 0.048	0.69± 0.040	0.70± 0.074	0.64± 0.036	0.53± 0.044
DZIBU	0.33± 0.024	0.047± 0.033	0.58± 0.042	0.56± 0.032	0.52± 0.038	0.40± 0.048	0.42± 0.024	0.26± 0.034	0.10± 0.026
	(21)	(23)	(31)	(23)	(30)	(42)*	(54)*	(59)*	(81)*
IBU	0.38± 0.012	0.42± 0.020	0.58± 0.037	0.55± 0.030	0.50± 0.038	0.45± 0.044	0.40± 0.029	0.37± 0.022	0.25± 0.024
	(9)	(31)	(31)	(25)	(32)	(34)	(43)*	(42)*	(53)*

Mean rat paw edema (ml)±S.E for the test compound (DZIBU) and the standard, ibuprofen (IBU) at the dose level of 0.5 mmol/kg.p.o daily for 10 d. (n=6) *P<0.05, significantly different from control (Student's t test). % antiinflammatory activity is shown in brackets.

scored separately. Aspirin was used as positive control.

A novel method of synthesizing curcuminoids has been successfully developed by which different 1,7-substitutions in the curcuminoid is possible unlike the method of Babu and Rajasekharan[3]. A pursuit towards developing an ideal antiinflammatory agent prompted us to consider the molecular manipulation of dehydrozingerone into an artifact of ibuprofen by combining both the molecule by Claisen-Dieckmann reaction. This is an easy and novel method of synthesizing various curcuminoids having different substituents on 1,7-position of 1,6-heptadiene-3,5-dione. Esters of α,β-unsaturated acids like ethyl ferulate and ethyl *p*-methoxycinnamate are allowed to react with dehydrozingerone to give the β-diketones. The structure of curcuminoids synthesized were confirmed by mp, elemental analyses and spectral data and were found to be identical with those reported in the literature[3]. The presence of 1,3-diketone stretching frequency was found as sharp absorption band near 1719 cm^{-1}. The conjugated α,β-unsaturated double bond with C=O is seen as absorption peak at 1610 cm^{-1}. The C-C-C stretching and

TABLE 4: INHIBITION OF GRANULOMA FORMATION, ULCEROGENICITY AND LD_{50} DETERMINATION.

Compound	Dose (mmol/kg)	Granuloma pellet test		Ulcer score#	LD_{50} (Oral)$ (mg/kg b.w.)
		Dry weight (mg) of granuloma ±S.E@	% Inhibition		
Control	-	33.47±1.86	0	0	-
DZIBU	0.5	21.33±1.81*	36.27	0	4950
IBU	0.5	20.80±1.40*	37.85	2	-

@ Test compound (DZIBU) and the standard ibuprofen (IBU) were administered orally for 7 d from the day of pellet implantation in albino rats (n=6). Dry weight of granuloma without pellet weight, values are mean±S.E *P<0.05, singificantly different from control (Student's t test). # Ulcer score of the test compounds as per the method of Cashin *et al.* $ LD_{50} value in mice after oral administration of single dose of test compound, calculated by graphic method of Miller and Tainter using probit plotted against log dose.

bending is observed at 1230 cm^{-1}. Unbound free phenolic OH stretching vibration is seen as a broad peak at 3429 cm^{-1}. The sharp singlet peak due to methylene proton of 1,3-diketone is observed at δ 3.63 in the ^1H NMR spectrum of DZIBU. The protons of α,β-unsaturated double bond is seen at δ 6.74. The strong evidence of the condensation reaction is obtained as M^+ peak at 380 m/z of mass spectrum corresponding to the molecular weight of the structure postulated.

The antiinflammatory activity of DZIBU was tested for acute, sub acute and chronic models using established methods. In carrageenan-induced rat paw edema method, DZIBU exhibited highly significant activity (76%) comparable to that of ibuprofen (73%) in equimole dose level[13]. The test compound showed same degree of antiinflammatory activity in adrenalectomised rats indicating that the antiinflammatory activity of this compound is not mediated via pituitary adrenal axis. The log-dose response studies brought excellent correlation. The regression equation obtained for DZIBU is A = 78 x log X- 157, (r = 0.94, n=5), where A is % antiinflammatory activity and X is dose in mmol/kg. DZIBU exhibited predominant activity against formaldehyde-induced arthritis and at 0.5 mmol/kg dose level, it reversed the disease symptoms and reduced periarticular swelling when compared with control group.

In chronic antiinflammatory testing model of cotton pellet-granuloma formation in albino rats the results are not that promising. DZIBU produced 30% inhibition of granuloma formation while ibuprofen exhibited 38% activity at an equimole dose level. The results support the general finding that NSAIDs usually exhibit low activity in this model unlike corticosteroids. The test compound did not induce gastrointestinal ulceration even at 1 mmol/kg dose level administered orally. The histopathological examination of gastric mucosa in acute treatment did not show presence of infiltration of neutrophils in the epithetial cells, inflammatory epithelial cells or ulceration. The preliminary biological studies indicate that DZIBU is potent antiinflammatory and analgesic agent without any ulcerogenic side effects.

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